Chapter 46
Mineralizable Nitrogen

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46.1 INTRODUCTION

Nitrogen (N) is generally the most common growth-limiting nutrient in agricultural production systems. The N taken up by crops is derived from a number of sources, particularly from fertilizer, biological N fixation and mineralization of N from soil organic matter, crop residues, and manures (Keeney 1982). The contribution of mineralization to crop N supply may range from <20 to >200 kg N ha^{-1} (Goh 1983; Cabrera et al. 1994) depending on the quantity of mineralizable organic N in the soil and environmental conditions (soil temperature and moisture) that control the rate of mineralization. Large amounts of mineralizable N can accumulate under grassland with the result that crops grown immediately after cultivation of long-term grass may derive much of their N from mineralization. In contrast, soils that have been intensively cropped often mineralize little N, leaving crops heavily dependent on fertilizer N.

Potentially mineralizable N is a measure of the active fraction of soil organic N, which is chiefly responsible for the release of mineral N through microbial action. Mineralizable N is composed of a heterogeneous array of organic substrates including microbial biomass, residues of recent crops, and humus. Despite a continuing research effort (Jalil et al. 1996; Picone et al. 2002), chemical tests that are selective for the mineralizable portion of soil N are not available and incubation assays remain the preferred way of estimating mineralizable N.

Stanford and Smith (1972) proposed a method to estimate potentially mineralizable N based on the mineral N released during a 30 week aerobic incubation of a soil:sand mixture under optimum temperature and moisture conditions. Although this procedure is regarded as the standard reference method, its main application is as a research tool because it is too time-consuming for routine use. Shortened versions of the aerobic incubation method have been found useful in evaluating soil N supplying power (Paul et al. 2002; Curtin and

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McCallum 2004), but these assays still take several weeks to complete and require considerable technical expertise.

An anaerobic incubation method for estimating mineralizable N was proposed by Keeney and Bremner (1966). This anaerobic (i.e., waterlogged soil) technique has significant practical and operational advantages over aerobic techniques in that the incubation period is relatively short (7 days) and the need for careful adjustment of soil water content is avoided. This assay is occasionally used for routine soil fertility testing by commercial laboratories. Although Keeney and Bremner (1966) reported good correlations between anaerobically mineralizable N (AMN) and plant N uptake under greenhouse conditions, subsequent work with field-grown crops has given mixed results (Thicke et al. 1993; Christensen et al. 1999).

46.2 POTENTIALLY MINERALIZABLE N

46.2.1 THEORY

In theory, potentially mineralizable N is the amount of N that will mineralize in infinite time at optimum temperature and moisture. It is estimated by incubating soil under optimal conditions and measuring N mineralized as a function of time by periodically leaching mineral N from the soil. Potentially mineralizable N is calculated using a first-order kinetic model:

$$N_{\min} = N_0(1-e^{-kt}) \tag{46.1}$$

where $N_{\min}$ is cumulative N mineralized in time t, N_0 is potentially mineralizable N, and k is the mineralization rate constant. This equation has two unknowns (N_0 and k), which are usually estimated by least-squares iteration using appropriate statistics software.

46.2.2 MATERIALS

1. Incubator capable of maintaining temperatures of up to 40°C (and humidity near 100% so that soils do not dry out during incubation).
2. Vacuum pump to extract leachate at ~ -80 kPa.
3. Leaching units to hold incubating soils. These can be purpose-made leaching tubes (Campbell et al. 1993), commercially available filter units (e.g., 150 mL membrane filter units; MacKay and Carefoot 1981), or Buchner funnels (Ellert and Bettany 1988; Benedetti and Sebastiani 1996).
4. Glass wool to make a pad ~6 mm thick at the bottom and 3 mm on top of the incubating sample.
5. Acid-washed, 20 mesh quartz sand.
6. 0.01 *M* $CaCl_2$ leaching solution (made from a stock solution of $CaCl_2$).
7. N-free nutrient solution containing 0.002 *M* $CaSO_4$, 0.002 *M* $MgSO_4$, 0.005 *M* $Ca(H_2PO_4)_2$, and 0.0025 *M* K_2SO_4 to replace nutrients removed from the soil during leaching.

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46.2.3 Procedure

1 Soils are usually air-dried and sieved before incubation, but field-moist soil may also be used. The N mineralization rate can be quite sensitive to sample pretreatment, particularly in the early phase of incubation (see Section 46.2.5).

2 Mix 15–50 g of soil with sand at a soil:sand ratio of 1:1 for medium-textured soils and 1:2 for fine-textured soils. It may be helpful to apply a light mist of water to prevent particle/aggregate size segregation during transfer to the leaching tubes.

3 Sand–soil mixture is supported in the leaching tube on a glass wool pad or by a sandwich of glass wool/Whatman glass microfiber filter/glass wool. A thin pad of glass wool is placed on top of the soil–sand mixture to prevent aggregate disruption when leaching solution is applied.

4 Native mineral N is leached using 100 mL of 0.01 *M* $CaCl_2$, applied in small increments (~10 mL) followed by 25 mL of N-free nutrient solution. The soil–sand mixture is initially allowed to drain naturally, then a vacuum (−80 kPa) is applied to remove excess water. Discard the first leachate.

5 Tubes are stoppered at both ends and placed in an incubator at 35°C. A hypodermic needle (38 mm, 16–18 gauge) is inserted in the bottom to facilitate aeration. Twice per week the top stopper is briefly removed to facilitate aeration.

6 Step 4 (leaching) is repeated every 2 weeks for the first 8–10 weeks of incubation and every 4 weeks thereafter. The collected leachate is filtered through a prewashed Whatman No. 42 filter paper and analyzed for NO_3- and NH_4-N.

7 Incubation can be terminated when cumulative N mineralized approaches a plateau. This usually occurs after about 20 weeks (see Section 46.2.5).

46.2.4 Calculations

Nonlinear least-square regression is the preferred statistical technique to estimate N_0 and k in the first-order kinetic model (Campbell et al. 1993; Benedetti and Sebastiani 1996). Rough estimates of N_0 and k are needed to initiate the calculation. We suggest an initial estimate of $k \sim 0.10$ per week (values normally between 0.05 and 0.20 per week) and N_0 can be assumed to be about 50% greater than cumulative mineralized N at the end of the incubation period (Campbell et al. 1993).

46.2.5 Comments

1 The most appropriate way of handling samples before incubation has not been established. Both air-dry soil and field-moist samples have been used. Where moist samples are to be used, they should be refrigerated (about 4°C) in the period between sampling and incubation. Campbell et al. (1993) recommend air-drying after collection, which may be appropriate in regions where soils become air-dry in the field. Air-drying can kill off part of the microbial biomass and rapid mineralization of this microbial-N will occur upon rewetting.

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The single-exponential model (Equation 46.1) may not adequately describe the initial flush of mineralization that occurs after rewetting (Cabrera 1993) and data for the first 2 weeks have sometimes been excluded when estimating N_0 (Stanford and Smith 1972). The degree of sample disturbance (e.g., fineness of sieving) may also influence the results. However, Stenger et al. (2002) found little difference in N mineralization (6 month incubation) between intact and sieved (<2 mm) soils.

2 An assumption implicit in Equation 46.1 is that there is only one pool of mineralizable N. This assumption is dubious as there is clear evidence for the existence of several forms of ''active'' N. There have been attempts to improve data fit by assuming two or three pools of mineralizable N (Deans et al. 1986). While a two pool (i.e., double exponential) model usually fits laboratory N mineralization data more precisely than a single-exponential model (Curtin et al. 1998), many workers consider the improvement insufficient to warrant its use for general purposes (Campbell et al. 1988).

3 Values of N_0 and k obtained by data fit to Equation 46.1 can vary depending on temperature, moisture content, and duration of incubation (Wang et al. 2003). The optimum temperature for N mineralization is often considered to be 35°C. Campbell et al. (1993) suggested that incubation at a lower temperature (e.g., 28°C) may result in a lag phase in N mineralization during the first 2 weeks of incubation. A lag phase may be exhibited by soils containing C-rich substrates (e.g., forest soils) where net N mineralization may initially be low because N immobilization predominates (Scott et al. 1998). Optimum soil moisture content is about field capacity (−5 to −10 kPa). The incubation time should, ideally, be at least 25 weeks (Ellert 1990). Values of N_0 tend to increase and k to decrease as incubation time is extended (Paustian and Bonde 1987; Wang et al. 2003). Cumulative N mineralized (N_{min}) typically increases asymptotically to reach a plateau after about 16–20 weeks of incubation (Campbell et al. 1993).

4 A problem inherent in fitting the first-order model to mineralization data is that there tends to be an inverse relationship between N_0 and k. It has been argued that to obtain values of N_0 that are truly indicative of the amount of mineralizable in the soil, k should be set to a standard value (e.g., 0.054 per week) (Wang et al. 2003). This approach minimizes the effect of incubation time on N_0 (values not affected by changes in incubation duration from 20 to 40 weeks; Wang et al. 2003).

46.3 SHORT-TERM AEROBIC INCUBATION

Short-term aerobic incubation techniques have the obvious advantage that a more timely estimate of mineralizable N can be obtained, and, since periodic leaching is not required, the labor requirement is reduced. Based on analysis of two data sets, Campbell et al. (1994) showed that N mineralized in the first 2 weeks of incubation was reasonably well related to N_0 in North American soils. However, this may not always be the case. Certain soils (e.g., forest soils with high C:N ratio; Scott et al. 1998) can immobilize substantial N during short incubation and net N mineralized in the short-term may not be closely related to N_0. Various short-term incubation assays have been proposed; they differ in incubation duration and temperature. Parfitt et al. (2005) reported that N mineralized in a 56 day aerobic incubation (25°C) was closely correlated with N uptake by legume-based pastures in New Zealand.

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Nitrogen mineralized in a 28 day aerobic incubation (20°C) was closely related to N uptake by a greenhouse-grown oat (*Avena sativa* L.) crop from 30 soils representing a range of management histories and parent materials (Curtin and McCallum 2004). Longer (56 vs. 28 days) incubations may give results that more accurately reflect N supply over a growing season, but may not be attractive where timeliness of results is an important consideration. Field rates of mineralization may be estimated by adjusting the basal value (i.e., the value determined by incubation under defined temperature and moisture conditions) using soil temperature and moisture adjustment factors (Paul et al. 2002).

The following procedure is based on the method used by Scott et al. (1998) and Parfitt et al. (2005).

46.3.1 Procedure

1. Weigh sieved (<4 or 5 mm), field-moist soil (equivalent to about 5 g of dry soil) into 125 mL polypropylene containers (use of field-moist soil is recommended to avoid the flush of mineralization that occurs when air-dry soil is rewetted; however, air-dry samples may be appropriate for semiarid soils).

2. Add water to adjust the soil water content so that it is equivalent to −10 kPa. Soil water content at −10 kPa is normally determined from tension table measurements on a separate sample.

3. Cover containers with polyethylene (30 μm) held in place with rubber bands and place in plastic trays containing water, enclosed in large polyethylene bags (to maintain high humidity).

4. Incubate at the desired temperature (20°C to 30°C) for the required time period (e.g., 28 or 56 days).

5. Measure mineral N (NO_3- plus NH_4-N) at the end of incubation by extraction with 2 *M* KCl. Mineral N in the soil before incubation is determined by extracting a separate sample with KCl.

6. Mineralized N is calculated by subtracting initial mineral N from that determined at the end of the incubation.

46.4 ANAEROBIC INCUBATION

This technique offers important operational and practical advantages that make it more suitable for routine use than aerobic incubation. The incubation period is relatively short (7 days); the same volume of water is added to all soils regardless of water holding capacity; and NH_4-N only needs to be measured because NO_3-N is not produced under anaerobic conditions.

46.4.1 Procedure

1. Weigh 5 g of sieved (<4 or 5 mm) soil into a 50 mL plastic, screw-cap centrifuge tube. Add 10 mL of distilled water to submerge the soil, stopper the tube, and place in a constant temperature (40°C) cabinet/incubator for 7 days.

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2 Remove tube from incubator and add 40 mL of 2.5 *M* KCl (after dilution with water in the sample, final KCl concentration is 2 *M*). Mix contents of tube, centrifuge at 1900 *g*, and filter the supernatant (prewashed Whatman No. 42).

3 Determine NH_4-N in the supernatant. Measure the amount of NH_4-N in the soils before incubation by extracting a separate sample with KCl. Mineralized N is estimated by deducting this preincubation NH_4-N value from the amount measured in the incubated sample.

46.4.2 Comments

1 Since most arable soils do not contain appreciable NH_4-N, it may be possible to dispense with the initial NH_4-N measurement; however, preliminary checks should be carried out to verify that native NH_4-N is negligible (Keeney 1982).

2 Sample preparation has not been standardized; air-dry and field-moist soils are commonly used to measure AMN. Larsen (1999) suggests that pretreatment (air-drying, freezing) can have a strong effect on AMN and he recommends the use of fresh, field-moist soil.

3 Although AMN is correlated with the N mineralized in an aerobic incubation, the relationship is often not very close (Curtin and McCallum 2004).

4 To be useful as part of a fertilizer N recommendation system, an empirical calibration of AMN against crop performance under local field conditions is recommended (Christensen et al. 1999).

46.5 CHEMICAL INDICES OF NITROGEN MINERALIZATION CAPACITY

Because of the time requirement of the biological assays described above, chemical tests have been evaluated as possible surrogates. Chemical procedures have the advantage that they can be more rapid and precise than biological (incubation) assays but, to date, no extractant has been capable of simulating the microbially mediated release of mineral N that occurs in incubated soil. Most chemical tests are relatively simple in their mode of action, i.e., they selectively extract a particular form or forms of N. On the other hand, mineralization is a complex microbial process comprised of subprocesses that release (gross mineralization) and consume (immobilization) mineral N. Net N mineralization, as measured in incubation assays, is the balance between the processes of gross N mineralization and N immobilization. Chemical tests that select for labile fractions of soil N have potential in estimating gross N mineralization (Wang et al. 2001). However, predicting net N mineralization based on a chemical extraction test is more problematic because such tests cannot account for N immobilization.

Although many chemical tests for N availability have been proposed (listed by Keeney 1982), none of them has been adopted for general or routine use in soil fertility evaluation. Perhaps the chemical test that has attracted most attention in the past decade is hot 2 *M* KCl extraction, which causes hydrolysis of some organic N to NH_4 (Gianello and Bremner 1986). Despite some encouraging observations (Gianello and Bremner 1986; Jalil et al. 1996; Beauchamp et al. 2004), the performance of the test has not been consistent overall

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(Wang et al. 2001; Curtin and McCallum 2004). Work on chemical test development and evaluation is continuing (e.g., Mulvaney et al. 2001; Picone et al. 2002). However, there is presently no agreement among researchers on which of the available soil N tests has the most potential to serve as a predictor of soil N supplying power and, until scientific consensus emerges, it would be unwise to recommend any test for general use.

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Chapter 47
Physically Uncomplexed Organic Matter

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47.1 INTRODUCTION

Physically uncomplexed organic matter is composed of particles of organic matter (OM) that are not bound to soil mineral particles and can be isolated from soil by density (using heavy liquids) or size (using sieving) fractionation. It is separated from soil on the premise that the association of organic matter with primary soil (mineral) particles alters its function, turnover, and dynamics in the soil environment. Uncomplexed organic matter has been isolated to study the form and function of soil organic constituents and to assess the impacts of land use, management, and vegetation type on carbon (C) and nitrogen (N) turnover and storage (Gregorich and Janzen 1996; Gregorich et al. 2006, and references therein). It has been separated and evaluated in studies pertaining to nutrient availability (Campbell et al. 2001), decomposition of plant residues (Magid and Kjærgaard 2001), physical protection of soil organic matter (Beare et al. 1994), and aggregation processes (Golchin et al. 1994).

Physically uncomplexed organic matter is a mixture of plant, animal, and microorganism parts at different stages of decomposition, and includes pollen, spores, seeds, invertebrate exoskeletons, phytoliths, and charcoal (Spycher et al. 1983; Baisden et al. 2002). Light fraction (LF) organic matter and particulate organic matter (POM) are the most commonly isolated forms of physically uncomplexed organic matter, though they differ in amount and their chemical characteristics. In this chapter, LF is defined as the organic matter recovered when soil is suspended in a heavy solution (i.e., heavier than water) of a known specific gravity, most often in the range of 1.6–2.0 (Sollins et al. 1999). In contrast, POM is defined as the organic matter recovered after passing dispersed soil through a sieve with openings of a defined size, normally between 250 and 53 μm in diameter. The POM has been isolated by size alone (e.g., >53 μm), or by a combination of size and density fractionation procedures (see Cambardella and Elliott 1992).

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The proportion of total soil C and N accounted for in physically uncomplexed organic matter can be substantial. Based on a review of more than 65 published papers, Gregorich et al. (2006) showed that for agricultural mineral soils, the amount of soil C and N accounted for in POM is usually much greater than that in the LF. On average, POM (50–2000 μm diameter) accounted for 22% of soil organic C and 18% of total soil N. In contrast, LF organic matter (specific gravity <1.9) accounted for 8% of soil organic C and 5% of total soil N. Limited work has been done on the phosphorus (P) or sulfur (S) content of LF organic matter, but research has shown that less than 5% of soil organic P resides within LF (Curtin et al. 2003; Salas et al. 2003).

The C:N ratio of physically uncomplexed OM is usually wider than that of whole soil, but narrower than that of plant residue. The C:N ratios of LF organic matter tend to narrow as specific gravity increases, ranging from 17 to 22 for specific gravities of 1.0–1.8 and from 10 to 17 for specific gravities of 1.8–2.2 (Gregorich et al. 2006). The relatively wide C:N ratio of LF extracted at low specific gravity (<1.8) reflects the dominant influence of plant constituents (e.g., lignin), whereas at a higher specific gravity the isolated material contains more mineral particles with adsorbed OM. Gregorich et al. (2006) also showed that there is a positive log–linear relationship between the mean size of POM fractions and their C:N ratio. In general, the variation in C:N ratios of larger size fractions is considerably greater than the variation in C:N ratios of smaller size fractions, which is consistent with the findings of Magid and Kjærgaard (2001).

The LF is usually isolated using liquids of a defined specific gravity, most often in the range of 1.6–2.0 (Sollins et al. 1999). POM has been isolated by size alone, or by a combination of size and density fractionation procedures (see Cambardella and Elliott 1992). We present two methods of separating uncomplexed organic matter in this chapter: (1) wet sieving of soil dispersed in a solution of sodium hexametaphosphate to isolate sand-sized (>53 μm) POM and (2) suspension of dispersed soil in a solution of sodium iodide (NaI) at a specific gravity of 1.7 to isolate the LF organic matter. A size of >53 μm is recommended for separating POM because, as a cutoff for the sand fraction in particle size analysis, it has been routinely used in POM studies (Gregorich et al. 2006). The 1.7 specific gravity recommended for isolating LF is in accord with early studies that indicated that this density separated most organomineral and mineral particles from decaying plant residues (Ladd et al. 1977; Scheffer 1977; Ladd and Amato 1980; Spycher et al. 1983).

47.2 PARTICULATE ORGANIC MATTER

Uncomplexed organic matter isolated by size is usually referred to as ''particulate organic matter'' (Cambardella and Elliott 1992) but has also been referred to as ''sand-size organic matter'' or ''macroorganic matter'' (Gregorich and Ellert 1993; Wander 2004). It is isolated by dispersing the soil and collecting the sand-sized fraction on a sieve. Where soils are first passed through a 2 mm sieve, the POM recovered on a 53 μm sieve can be defined as ranging in size from 53 to 2000 μm in diameter and as such represents a quantifiable component of the whole soil organic matter.

47.2.1 Materials and Reagents

1 A sieve with 2 mm openings.

2 Reciprocating or end-over-end shaker and 200–250 mL bottles or flasks with leakproof lids.

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3 Sodium hexametaphosphate solution, 5 g L^{-1} $(NaPO_3)_6$.

4 Sieves with 53 μm openings, 10 cm diameter (or larger), placed on top of the polypropylene funnel (larger diameter than sieve) supported by a ring clamp on a laboratory stand.

5 Tall-form beakers of 1 L capacity may be useful to collect the non-POM, silt + clay suspension that is washed through the sieve.

6 A large bottle of distilled water and a spatula or rubber policeman to ensure that the entire silt + clay fraction passes through the sieve.

7 Drying oven.

47.2.2 Procedure

1 Pass field-moist soil through a sieve with 2 mm openings and discard any residues retained on the sieve. Air-dry the soil.

2 Determine the soil water content by oven drying a subsample (5 g) of the soil at 105°C.

3 Weigh 25 g of air-dried soil into each bottle, dispense 100 mL of the sodium hexametaphosphate solution into each bottle, cap the bottles, and shake overnight (e.g., 16 h).

4 Pour the suspension onto the 53 μm sieve using small aliquots of water to rinse the soil from the bottle.

5 Wash the silt + clay-sized fraction, which includes mineral and fine organic matter through the sieve using a fine jet of water from the wash bottle and gently crushing any aggregates with a rubber policeman. The POM (i.e., sand + large particles of organic matter) is retained on the sieve.

6 Rapid drying can be achieved by first oven drying (≈1 h at 40°C) the POM directly on the 53 μm sieves before transferring the POM to a beaker or similar container for final oven drying at 60°C overnight. *Note*: Place a small tray under the sieve to catch any POM that may fall through the openings. Use a spatula or paintbrush to carefully remove the POM from the sieve, taking care to recover all of the sieve contents; record the dry weight of this material.

7 Use a mortar and pestle to grind and homogenize the oven-dry POM to pass through a sieve with 250 μm openings. Determine the concentrations of C, N, and other elements of interest.

47.2.3 Comments

1 Soils are usually air-dried before dispersion to remove the effects of variations in water content. Excessive abrasion of the soil during sample preparation or dispersion can result in fragmentation of the larger particles of organic matter and

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thereby lower the recovery of POM. Agents other than sodium hexametaphosphate have been used to disperse the soil before wet sieving (e.g., sonication, shaking with glass beads). In all cases, care should be taken to ensure that the amount of energy used to disperse the soil does not affect the quantity of POM recovered (Oorts et al. 2005).

2 POM may also be recovered by washing the sand-sized material from the sieve into preweighed drying tins using a wash bottle, evaporating overnight, and then oven drying at 60°C. If this is done, care should be taken to ensure that exposure of POM to water at high temperatures (during drying) for extended periods does not alter its chemical composition (e.g., through dissolution of C or nutrients).

3 It is generally assumed that any organic matter bound to the sand contributes relatively little to the carbon and nutrient concentrations measured in the material. However, where it is important to determine the dry weight of sand-free POM or to isolate POM from sand for other analyses, the sand-sized organic matter may be resuspended in a heavy solution to complete a further density separation of the organic matter (e.g., Cambardella and Elliott 1992). If this is done, care should be taken to ensure that the heavy liquid can be washed free from the POM before further analyses are undertaken.

4 Magid and Kjærgaard (2001) advocated the fractionation of POM in studies of residue decomposition. In these cases, it is sometimes useful to isolate different size classes of POM by placing a nest of sieves (e.g., 1000 and 250 μm) on top of the 53 μm sieve (Oorts et al. 2005).

47.3 LIGHT FRACTION ORGANIC MATTER

Light fraction organic matter can be isolated from much of the mineral soil by suspending the soil in a dense liquid and allowing the heavy fraction to settle while the LF floats to the surface. Density fractionation is based on the premise that the lighter soil particles, comprising mainly of freshly added, partially decomposed, and less humified organic matter, are more labile and reactive than heavier particles, which have variable amounts of adsorbed humified organic matter. The LF organic matter is separated by shaking the soil in a solution of NaI (specific gravity = 1.7) and allowing the soil mineral particles to settle for 48 h before recovering the suspended LF organic matter.

47.3.1 Materials and Reagents

1 A sieve with 2 mm openings.

2 Reciprocating or end-over-end shaker, plastic or glass bottles with lids and tall, narrow beakers (at least 250 mL capacity) with rubber stoppers. The shaker action and speed (revolutions or cycles per minute) and the flask orientation and geometry should be recorded, as these variables may influence the degree of soil dispersion.

3 Sodium iodide solution with a specific gravity of 1.7. Slowly add 1200 g of NaI to 1 L of water in a large beaker, while stirring and heating the solution on a

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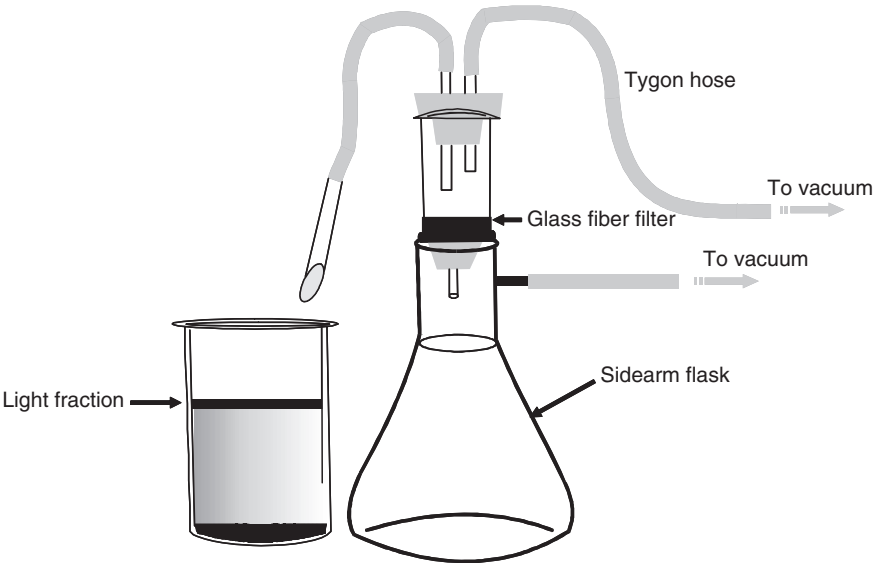

FIGURE 47.1. Vacuum filtration unit with a sidearm flask used to isolate light fraction organic matter.

magnetic mixer. After NaI is dissolved, cool the solution to room temperature and, with a hydrometer, adjust the specific gravity of the solution to 1.7. About 90 mL of solution, containing about 84 g of NaI, is required to separate the LF from each 25 g soil sample.

4 Aspiration unit (see Figure 47.1) consisting of a vacuum hose with a disposable pipette tip cut at a 45° angle to aspirate the LF; a fritted glass filter support; a detachable funnel; a clamp to attach the funnel to the top of the flask and two large (1 L) sidearm flasks, one to collect the dense solution for reuse and the other to collect water washings for discard. Membrane filters (e.g., 0.45 μm Millipore filters) made of nylon or ''quantitative'' filters designed for easy recovery of the LF on the filter may be used without contaminating the LF with filter-derived C.

5 Three wash bottles, one containing the NaI solution (specific gravity = 1.7), one containing 0.01 *M* $CaCl_2$, and one containing distilled water.

47.3.2 Procedure

1 Pass field-moist soil through a sieve with 2 mm openings and discard any residues retained on the sieve. Air-dry the soil.

2 Determine the soil water content by oven drying a subsample (5 g) of the soil at 105°C.

3 Weigh 25 g of soil into each bottle; dispense 50 mL of the NaI solution into each bottle; cap the bottle and shake on a reciprocating shaker for 60 min. Longer shaking times may be required when less vigorous shaking is used.

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4 Remove the lids from each bottle; pour the contents of each bottle into a 200 mL beaker using the wash bottle containing NaI to wash the soil from the lids and bottles into the beakers.

5 Allow the beakers to stand on the laboratory bench at room temperature for 48 h.

6 Aspirate the LF organic matter from the surface of each beaker (about the top 25 mL) into the filter unit, apply a suction, and collect the filtrate (specific gravity $= 1.7$) for reuse. Remove enough of the dense liquid to wash the LF organic matter from the vacuum hose.

7 Without disturbing the clamp or filter, transfer the filter unit from the sidearm flask containing dense solution filtrate (for reuse) to the sidearm flask that will be used to collect the washings. Use the wash bottle containing the $CaCl_2$ to wash any LF from the walls of the vacuum flask and funnel to the filter paper. Use about 75 mL of $CaCl_2$ solution followed by 75 mL of distilled water (at least 150 mL in all) to wash the NaI from the LF organic matter. ($CaCl_2$ will help prevent the clogging of the filter.) Discard the wash water (filtrate), but keep the NaI from the first flask for reuse.

8 Remove the filter and wash the LF into preweighed drying tins. Place tins in the oven at 60°C to obtain the dry weight of the LF.

9 If the soil contains large amounts of plant residue (e.g., forest soils), it may be necessary to repeat the procedure. If so, repeat the LF separation using the NaI solution remaining in step 6 above. First add enough fresh (or filtered) NaI solution (specific gravity of 1.7) to bring the volume to about 50 mL, resuspend the soil, and repeat steps 4–8 described above.

10 Combine the dried LF organic matter recovered from the two separations and use a mortar and pestle to grind this fraction to pass through a sieve with 250 μm openings. Determine the concentrations of C and N (or other elements of interest) in the LF using standard methods.

47.3.3 Comments

1 Various compounds have been used to produce dense solutions to isolate LF organic matter (see Gregorich and Ellert 1993). We recommend the use of NaI as it is less expensive and less toxic than most alternative media, is widely available, and can be used to make solutions with densities up to 1.9 g cm^{-3} at 25°C. Organic solvents have been used to fractionate soils on the basis of density, but these are not recommended because of problems with toxicity, C contamination, and coagulation of suspended particles (Gregorich and Ellert 1993; Sollins et al. 1999). Sodium metatungstate ($Na_6(H_2\ W_{12}\ O_{40})$, Aldrich Chemical Co., Milwaukee, Wisconsin) has been used to prepare solutions with specific gravities up to 3.1 at 25°C (Plewinsky and Kamps 1984). It is considered to be unreactive and solubilizes relatively small amounts of C (Sollins et al. 1999). Colloidal silica (Ludox TM40) has been used to make solutions with densities up to 1.37 g cm^{-3}, but they have a high pH (e.g., ~pH 9) and so may extract substantial amounts of humic materials.

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2 In addition to density, several solution properties (e.g., viscosity, surface tension, dielectric constant) may influence the results of density fractionations. For example, the apparent density of LF organic matter will depend on the extent to which the dense solution occupies the cavities in the particles, which in turn depends on the surface tension of the solution.

3 The density of soil particles reflects the ratio of organic materials to mineral particles (Sollins et al. 1999), and small variations in the specific gravity of the heavy liquid can result in large differences in the quantity of C (Richter et al. 1975) and C:N ratio (Gregorich et al. 2006) of the organic matter recovered. Our recommended density of 1.7 g cm^{-3} is within the range used by most researchers (Gregorich et al. 2006). To determine the most appropriate density to use in specific cases, Sollins et al. (1999) recommended undertaking sequential LF separations using solution densities ranging from 1.2 to 1.9 g cm^{-3} and analyzing the fractions obtained for ash content, C content, and C:N ratio. They contend that the optimum density for separating a biologically relevant LF is that above which the ash content of the LF increases substantially or the C:N ratio decreases markedly. It is often helpful to examine the LF under a stereomicroscope to determine the extent of any mineral soil contamination and identify biological constituents of the fractions.

4 The mass of solute required to attain a predetermined specific gravity can be computed from a measure of the solute concentration at a specific temperature. Concentration is expressed as mass fraction, because the mass, rather than volume, of solution components is additive:

$$F = S/(S + L) \tag{47.1}$$

$$S = FL/(1 - F) \tag{47.2}$$

$$S = F\ SG_{sol'n}V_{sol'n} \tag{47.3}$$

where F is the mass fraction, (i.e. mass of solute [Nal] expressed as proportion of mass of solute plus mass of solvent [water]), S the solute mass (g), L the solvent mass (g), $SG_{sol'n}$ the specific gravity of solution, and $V_{sol'n}$ the volume of solution (cm^{-3}). The mass fractions (F) for specific gravities of 1.6, 1.7, 1.8, and 1.9 are 0.51, 0.55, 0.60, and 0.64, respectively. For example, to achieve a Nal solution with a specific gravity of 1.8, where $F = 0.60$, then according to Equation, 47.2, 950 g of water requires 1425 g of Nal (final solution volume ~$(950 + 1425)/1.8$ or 1319 cm^3). Alternatively, Equation 47.3 indicates that 1425 g of Nal is required to prepare 1319 mL of a solution at a specific gravity of 1.8.

5 Centrifugal force (e.g., 1000 *g*) can be used to quickly separate the light and heavy fractions in the above procedure instead of leaving the beakers to stand on the laboratory bench (at 1 *g*) and will allow for more rapid processing of the samples. Use of centrifuge tubes also allows greater vertical separation of the light and heavy fractions and for narrower solution/soil ratios compared to those in the method described above; if the solution/soil ratio is decreased further than 2:1, some of the uncomplexed organic matter could get entrapped within the heavy

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fraction during the fractionation procedure. Therefore, if centrifugation is used, it is recommended that the heavy fraction be resuspended and the separation procedures (see step 9 in Section 47.3.2) repeated at least two or three times.

6 It is often useful to determine the C and nutrient content (e.g., N) of the whole soil so that the LF-C or -N can be expressed as a percentage of the whole soil C or N.

47.4 IMPORTANT CONSIDERATIONS

47.4.1 Calculation of Results

The mass of LF organic matter can be expressed as a percentage of the whole soil on a dry weight basis; however, it should be noted that the LF may contain a small amount of mineral soil contaminants that may result in an overestimation of the LF mass. To calculate the proportion of whole soil C in the POM or LF:

$$\text{fraction C/whole soil C} = [\text{fraction}_{dw} \times (\text{POM C or LF C})]/\text{whole soil C} \qquad (47.4)$$

where fraction C/whole soil C is the proportion of whole soil C in the POM or LF, fraction_{dw} is the dry weight of sand-sized or LF organic matter (g fraction/g whole soil), POM C or LF C is the C concentration in the POM or LF sample (g C/g fraction_{dw}), and whole soil C is the C content of the whole soil (i.e., g whole soil C/g whole soil).

Corrections for ash in LFs may help to account for the presence of light minerals or phytoliths. The ash content of the LF can be determined by weighing subsamples in a muffle furnace for 4 h at 550°C before and after ignition.

47.4.2 Losses during Fractionation

When first applying these procedures, it may be useful to determine the recovery efficiency and identify where losses may occur in the fractionation procedure. However, if care is taken with these procedures, it is probably not necessary to determine the recovery efficiency on a regular basis. The mass or organic C content of the whole soil can be compared with the sums of mass or C content in the various fractions to ensure that losses during the fractionation do not introduce appreciable bias. To calculate a mass balance, it is necessary to recover the silt + clay fraction in the sieving method or the heavy fraction in the flotation method. Calcium chloride (e.g., 20 mL of 3 *M* $CaCl_2$) or another flocculating agent can be added to the suspension passing the 53 μm sieve to recover the silt + clay associated organic matter. After the supernatant is siphoned off, the slurry left in the bottom of the beaker can be transferred to containers that are suitable for freeze drying. In the density separation method, the heavy fraction can be recovered by siphoning off the dense solution, and repeated resuspension in wash water followed by centrifugation and aspiration of the supernatant. When the heavy fraction fails to form a stable pellet (usually after two to three washings), it can be frozen in the centrifuge tubes and freeze dried.

47.4.3 Bioassay of the Light Fraction

The type of heavy solution used in separating the LF from whole soil may have deleterious effects on the viability of certain microbial populations and their activities, alter the decomposibility of LF organic matter, or cause complexation with the LF organic matter.

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Magid et al. (1996) observed that C mineralization from LF was enhanced when isolated with silica suspension and retarded when separated using sodium polytungstate. We recommend that any studies involving bioassays of the LF also include a thorough evaluation of possible contamination by the media and any resulting effects on decomposition.

47.4.4 Contamination of Uncomplexed Organic Matter with Charcoal or Mineral Soil

Physically uncomplexed organic matter can contain charcoal and its presence could substantially affect the chemistry and turnover of this organic matter. Charcoal has been detected using microscopic techniques in the LF and POM fractions in many soils (Spycher et al. 1983; Baisden et al. 2002). Where investigators are interested in LF or POM as a measure of ''young'' or actively cycling organic matter, removal of charcoal may be important to accurately estimate the size, nutrient content, and turnover of this fraction. However, there are no known standard procedures for correcting for the charcoal content of uncomplexed organic matter.

Given its operational definition, the POM (>53 μm) fraction of soil often contains a high proportion of sand that should be removed by density separation if a measure of the POM mass is required. Depending on the method of separation used, LF organic matter may also contain a small amount of mineral soil contaminants that may contribute some older and probably less labile, mineral-associated organic matter to what is measured in the LF.

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Chapter 48
Extraction and Characterization of Dissolved Organic Matter

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48.1 INTRODUCTION

Dissolved organic matter (DOM) represents a relatively small fraction of the total organic matter in soil (0.04%–0.2%; Zsolnay 1996). However, because of its mobility and presumed labile nature, DOM is often perceived as the most active fraction of soil organic matter. Research in the past 20 years has shown that DOM can play an important role in a number of key soil processes including the transport of nutrients (Murphy et al. 2000; Michalzik et al. 2001), organic contaminants and metals in the soil profile (Herbert and Bertsch 1995; Zsolnay 1996), replenishment of C at depth (Michalzik et al. 2001; Guggenberger and Kaiser 2003), and as a substrate for microbial activity (Burford and Bremner 1975; McGill et al. 1986; Chantigny et al. 1999; Marschner and Kalbitz 2003).

Dissolved organic matter is operationally defined as the organic matter present in solution that can pass through a 0.45 μm filter (Thurman 1985), though other pore sizes have sometimes been used for specific purposes (Herbert and Bertsch 1995). Various approaches have been used to obtain soil solution samples and these have different implications for the amount and composition of the DOM collected (Zsolnay 1996, 2003; Hagedorn et al. 2002, 2004). Much of the research on soil DOM has focused on temperate forest ecosystems (Zsolnay 1996; Kalbitz et al. 2000), where DOM is most often measured from soil solution samples

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collected *in situ* with zero-tension lysimeters. Other techniques involving tension lysimeters, suction cups, and centrifugation have also been used (Herbert and Bertsch 1995; Titus and Mahendrappa 1996). In grasslands and arable soils extraction of DOM with aqueous solutions is more common than the collection of soil solution *in situ* (Zsolnay 1996; Chantigny 2003) partly due to the frequent disturbances caused by management practices in agricultural soils which may interfere with lysimeter equipment. The ''soluble'' soil organic matter extracted with low-ionic strength aqueous solutions is often called water-extractable organic matter (WEOM), and is considered an acceptable surrogate to soil solution DOM collected *in situ* (Herbert and Bertsch 1995; Zsolnay 2003). Soil organic matter can also be extracted with high-ionic strength aqueous solutions. This procedure extracts both soil DOM and some additional organic matter desorbed during the extraction process; it therefore cannot be used as a surrogate to soil solution DOM collected *in situ*. However, soil organic matter extracted with high-ionic strength aqueous solution appears to be enriched in easily biodegradable compounds (Guggenberger et al. 1989; Novak and Bertsch 1991; Hagedorn et al. 2004), which could explain why it is considered to influence soil microbial biomass (e.g., McGill et al. 1986; Liang et al. 1998) and microbial processes such as denitrification (e.g., Burford and Bremner 1975; Lemke et al. 1998), soil respiration/C mineralization (e.g., Gregorich et al. 1998; Chantigny et al. 1999), and N mineralization (e.g., Appel and Mengel 1993; Murphy et al. 2000).

Dissolved organic matter is an expression borrowed from aquatic sciences (Thurman 1985; Zsolnay 2003). In soil science, the term dissolved may refer to organic matter present in any solution, including soil extracts. This might explain the confusion perceived in the scientific literature pertaining to the definition of soil DOM. A clear definition and distinction among the various procedures used to ''dissolve'' soil organic matter would be useful to soil researchers. For convenience, DOM is used in this chapter as a general term, and the most commonly used procedures to obtain soil DOM are classified into three distinct categories: soil solution, water-extractable, and salt-extractable organic matter. General procedures for collection and analysis of each category of soil DOM are presented. Selected procedures for analyzing C and N concentration, key spectroscopic and chemical properties, and biodegradability are also given.

48.2 COLLECTION OF SOIL DISSOLVED ORGANIC MATTER

48.2.1 Soil Solution Organic Matter

Careful selection of procedures to collect soil solution organic matter (SSOM) is needed to ensure that they are most appropriate to the research questions being addressed. Several approaches and devices have been proposed to collect soil solution; the most common involves the *in situ* use of lysimeters or suction cups (Heinrichs et al. 1996; Titus and Mahendrappa 1996; Ludwig et al. 1999), or the centrifugation of field-moist soil samples (reviewed by Zsolnay 1996). The selection of a procedure must be carefully made because different approaches may collect different fractions of the soil solution (Raber et al. 1998; Zsolnay 2003). For instance, zero-tension lysimeters collect freely draining soil solution, whereas tension lysimeters (e.g., suction cups) and centrifugation can collect solution located in smaller soil pores. The possible interferences of lysimeter surfaces with soil solution DOM have been addressed by Guggenberger and Zech (1992), Jones and Edwards (1993), Marques et al. (1996), Wessel-Bothe et al. (2000), and Siemens and Kaupenjohann (2003).

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Further details on the collection of soil solution are given in Chapter 17. In any case, soil solution samples must be filtered at 0.45 μm prior to analysis.

48.2.2 Water-Extractable Organic Matter (Adapted from Zsolnay 1996; Kalbitz et al. 2003)

Water-extractable organic matter has been proposed and used as a surrogate to soil solution collected *in situ* (Herbert and Bertsch 1995; Zsolnay 1996). The procedure was developed to minimize or avoid the release of OM through physical disruption of the soil structure and its desorption from exchange sites (Zsolnay 2003).

Materials and Reagents

1. Polypropylene centrifuge tubes (50 mL) or centrifuge bottles (250 mL)
2. Glass rod
3. Pure deionized water or 5 m*M* $CaCl_2$ solution
4. Centrifuge (optional)
5. Glass vacuum filter unit or stainless steel pressure filter unit
6. 0.4 μm polycarbonate filter that fits the filter unit
7. 125 mL Erlenmeyer flasks to support the filter unit (with sidearm if a vacuum filter unit is used)
8. Vacuum pump or other vacuum/pressure system
9. Storage vials of the required volume capacity (glass vials should be preferred; if freezing is necessary then plastic vials should be used)

Procedures

1. Place 5 g of mineral soil (dry mass basis) into a 50 mL centrifuge tube, or 5 g of organic soil (dry mass basis) into a 250 mL centrifuge bottle. Mineral soil samples should be thoroughly mixed or sieved at <6 mm to provide a representative subsample. Extraction procedures should be performed on field-moist soils and started as soon as possible after sampling as WEOM concentration and/or composition may change when field-moist soils are stored at cool temperatures for several days (Chapman et al. 1997b; Kaiser et al. 2001).
2. Add 10 mL of 5 m*M* $CaCl_2$ solution to the mineral soil, or 50 mL of deionized water to the organic soil. Gently stir with a glass rod to make a homogeneous slurry; stir for about 1 min for mineral soil extraction; for organic soil, let stand at 4°C for 24 h and occasionally stir (3–4 times) the slurries with the glass rod. Stirring must be as gentle as possible to avoid significant desorption of soluble materials.

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3 Centrifuge at 12,000 *g* for 10 min. This step is optional and is aimed at reducing clogging problems of filters caused by colloidal particles.

4 Filter the slurry (not centrifuged) or supernatant (centrifuged) through the vacuum or pressure filter unit equipped with a 0.4 μm polycarbonate filter.

5 Transfer the filtrate into a glass vial and store at 4°C if analyzed within 2 days; store the filtrate in a plastic vial at −20°C for longer periods.

Comments

1 A 1:2 soil:solution ratio for mineral soils and 1:10 ratio for organic soils are recommended as wider ratios may increase WEOM content by favoring organic matter desorption (Chapman et al. 1997a; Zsolnay 2003).

2 Proposed extraction times (1 min for mineral soil; 24 h for organic soil) have been tested by Zsolnay (1996) and Kalbitz et al. (2003), respectively. However, extraction times of 1 to 5 min were found to have a minimal influence on extraction efficiency of WEOM from mineral soils (Zsolnay 1996). Therefore, it is important to clearly indicate the extraction time used and to be consistent within each study.

3 If filtration is performed under vacuum (negative pressure), care must be taken that vacuum is not too high to avoid cavitation in the filtrate, which might modify the amount and nature of DOM; more details about this and other possible artifacts during DOM collection/extraction are reviewed by Zsolnay (2003).

4 Air-drying soil prior to extraction can increase the concentration of WEOM (Zsolnay et al. 1999; Kaiser et al. 2001), owing to microbial cell lysis and release of soluble components, or swelling of clays.

5 Use of deionized water is not recommended for extraction of mineral soils since it has a dispersive effect on soil aggregates and may favor organic matter desorption from mineral surfaces during extraction (Zsolnay 1996; Kaiser et al. 2001).

6 WEOM obtained with vigorous shaking (e.g., agitation on a reciprocal shaker) should not be considered an acceptable surrogate to soil solution collected *in situ* because the agitation may cause extraction of additional organic materials (Herbert and Bertsch 1995; Zsolnay 1996) of different nature (Zsolnay 2003; Hagedorn et al. 2004), likely due to aggregate disruption and/or abrasion of microbial cells. It then has more similarities with soluble organic materials obtained with the procedures given in the next section.

48.2.3 Salt-Extractable Organic Matter

The organic matter recovered from salt-solution extracts is also often referred to as ''soluble organic matter'' or ''water-soluble organic matter'' in the literature. Salt-extractable organic matter (SEOM) includes both SSOM and additional organic matter desorbed and/or dissolved

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during the extraction process (Zsolnay 1996, 2003; Hagedorn et al. 2004). For example, in a literature review Zsolnay (1996) reported SEOM (extraction with 0.5 *M* K_2SO_4 solution) values ranging from 29 to 127 μg g^{-1} dry soil in arable soils as compared to 8 to 13 μg g^{-1} dry soil for WEOM (extraction with either 4 m*M* $CaSO_4$ or 10 m*M* $CaCl_2$). Moreover, the additional material extracted appears to be more biodegradable than SSOM (Guggenberger et al. 1989; Novak and Bertsch 1991; Hagedorn et al. 2004). Therefore, SEOM should not be used as a surrogate to SSOM. Nevertheless, SEOM is often used as an estimate of organic matter readily available to soil heterotrophs (e.g., Burford and Bremner 1975; McGill et al. 1986; Murphy et al. 2000). The procedure given here is similar to that used for soil mineral N extraction (see Chapter 6).

Materials and Reagents

1. Polypropylene centrifuge bottles (250 mL)
2. Reciprocal shaker
3. 1 *M* KCl solution
4. Centrifuge (optional)
5. Glass vacuum filter unit or stainless steel pressure filter unit
6. 0.4 μm polycarbonate filter that fits the filter unit
7. 125 mL Erlenmeyer flask to support the vacuum filter unit (with sidearm if a vacuum filter unit is used)
8. Vacuum pump or other vacuum/pressure system
9. Storage vials of the required volume capacity (glass vials should be preferred; if freezing is necessary then plastic vials should be used)

Procedures

1. Place 20 g of soil (dry mass basis) in a centrifuge bottle. Mineral soil samples should be thoroughly mixed or sieved at <6 mm to provide a representative subsample. Soil samples should be extracted as soon as possible after soil sampling for the same reasons as given in Procedures, p. 619.
2. Add 100 mL of 1 *M* KCl solution. Agitate for 30 min on a reciprocal shaker (about 160 strokes per minute).
3. Centrifuge at 3,000 *g* for 10 min. This step is optional and is aimed at reducing clogging problems of filters caused by an excess of colloidal particles.
4. Filter the slurry (not centrifuged) or supernatant (centrifuged) through the vacuum or pressure filter unit equipped with a 0.4 μm polycarbonate filter.
5. Transfer the filtrate into a glass vial and store at 4°C if analyzed within 2 days; store the filtrate in a plastic vial at −20°C for longer periods.

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Comments

1 Extraction and measurement of SEOM in KCl extracts is proposed since this extraction procedure is routinely used to measure soil mineral N content. However, SEOM is also often measured in 0.5 *M* K_2SO_4 extracts, such as those taken from unfumigated soils used in measuring soil microbial biomass based on direct extraction procedures (Vance et al. 1987; see Chapter 49). Hot-water or hot KCl extractions are sometimes used and should not be considered equivalent to SSOM since heating of the soil-water slurry may dissolve more organic material than extraction at room temperature.

2 Air-drying of the soil prior to extraction can increase the amount of SEOM and may change its biodegradability if the additional extracted organic matter has different chemical characteristics. See the reviews by Zsolnay (1996; 2003) and Murphy et al. (2000) for more details about the various approaches used to obtain soil SEOM.

48.3 METHODS FOR CHARACTERIZING DISSOLVED ORGANIC MATTER

It is now widely accepted that DOM has a complex chemical composition and may contribute to a wide range of soil processes. In this section, we present details of relatively inexpensive and simple analytical methods for characterizing the chemical composition and assessing the biodegradability of DOM in soil solution (SSOM) and in soil extracts (WEOM and SEOM). Procedures for quantifying the total C, total N, specific UV absorbance, phenol, hexose, pentose, and amino acid content, and to assess DOM biodegradability are given below.

48.3.1 Carbon Concentration

Carbon is an important constituent of soil DOM. The C concentration in filtered soil solutions or extracts can be measured using wet chemical or automated combustion procedures. Quantification of C may be determined by UV-catalyzed wet oxidation followed by measurement of the evolved CO_2 using an infrared detector. However, C quantification by dry combustion at 700°C–800°C is now preferred since it can readily and more accurately measure inorganic and organic forms of dissolved C.

48.3.2 Nitrogen Concentration (after Cabrera and Beare 1993)

Researchers are often interested in determining the amount of nutrients present in organic forms in the soil solution or aqueous extracts. The organic N present in DOM can be measured by oxidation with potassium persulfate. At high temperature persulfate oxidizes organic N to NO_3^-, which can be measured by standard colorimetric methods (e.g., Cd reduction of NO_3^- to NO_2^-).

Materials and Reagents

1 Certified low-N potassium persulfate ($K_2S_2O_8$; EM Science, EM Industries, Inc. Gibbstown, NJ, USA).

2 Boric acid (H_3BO_4).

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3 Nanopure water (specific resistance of 17.8 megohm cm^{-1} or higher).

4 3.75 *M* NaOH solution: dissolve 150 g of NaOH pellets in 900 mL of nanopure water. Let the solution cool down to room temperature. Complete to 1 L with nanopure water.

5 Oxidative solution: dissolve 100 g of $K_2S_2O_8$ and 60 g of H_3BO_4 in 200 mL of 3.75 *M* NaOH solution. Complete to 2 L with nanopure water.

6 50 mL glass tubes equipped with Teflon-lined screw caps.

Oxidation Procedure

1 Measure mineral N (NO_2^--N + NO_3^--N + NH_4^+-N) concentration in the DOM sample using a standard procedure as proposed in Chapter 6.

2 Transfer/pipette 15 mL of DOM sample into a 50 mL glass tube and add 15 mL of the oxidative solution. Immediately close the tube with screw cap, agitate on a vortex for a few seconds, and weigh each tube.

3 Autoclave the loosely capped tubes for 30 min (121°C; 135 kPa).

4 Let cool down at room temperature and then tightly close the tubes. Weigh each tube: water loss during the autoclaving period is generally less than 3% of the initial volume and is used to correct for NO_3^- concentration measured in the oxidized solution.

5 Measure NO_3^- concentration in the oxidized solution using a standard procedure (see Chapter 6).

6 Organic N concentration is calculated as the difference between NO_3^--N concentration in the oxidized sample (Step 5) and the initial mineral N concentration in the nonoxidized sample (Step 1).

Comments

1 Mineral N concentration can be measured in both oxidized and nonoxidized samples with automated flow injection analysis (FIA) systems. Some FIA systems now offer the possibility for online UV-catalyzed persulfate oxidation of organic N, with simultaneous quantification of both the mineral and total dissolved N. The oxidation performance of those automated systems appears as good as the manual procedure presented here.

2 It should be noted that the method may oxidize some N_2, thus the volume of air in the test tubes should be as small as possible (Hagedorn and Schleppi 2000).

48.3.3 Specific UV Absorbance

The specific UV absorbance is an estimate of the concentration of aromatic compounds (Traina et al. 1990; Novak et al. 1992; Chin et al. 1994; Korshin et al. 1997) present in

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DOM sample. It has been shown to be positively correlated to the amount of XAD-8 adsorbable dissolved organic C (DOC) (Dilling and Kaiser 2002; Kalbitz et al. 2003) and phenol concentration as measured in the following section. In DOM samples from forest floor horizons, peat samples and A horizons, specific UV absorbance has been shown to be negatively correlated with DOM biodegradability, indicating that aromatic compounds are part of recalcitrant DOM (Kalbitz et al. 2003).

Materials and Reagents

1 1-cm path length quartz glass cuvettes

2 Spectrophotometer

Procedure and Calculations

1 Pour a sufficient amount of DOM sample into the quartz cuvette.

2 Read absorbance at 254–285 nm against blank.

3 Calculate specific UV absorbance by dividing the measured absorbance (in cm^{-1}) by the concentration of DOC (in mg L^{-1}) of the sample. The units of specific UV absorbance are thus given as L mg^{-1} C cm^{-1}.

4 Always report the wavelength used.

Comments

1 To avoid acidification and sparging of sample, specific UV absorbance should be carried out at ambient pH. Quartz glass cuvettes are to be used for this measurement as optical glass or plastic cuvettes may absorb some UV light. Make sure that the sample absorbance does not exceed the linear range of the instrument. If this is the case, dilute the sample with nanopure water.

2 Interferences may be caused by Fe^{2+}, NO_3^-, NO_2^-, and Br^-. However, the interference with NO_3^- can be minimized by performing the determination at 280 nm, and concentrations of NO_2^- and Br^- are generally too small to cause interference.

48.3.4 Phenols

Dissolved phenols, either monomers or oligomers, mainly derive from degradation of polyphenolic plant metabolites, such as tannins and lignin (Guggenberger et al. 1989). They are important metal-complexing agents, can bind proteins, interfere with the sorption of inorganic anions such as phosphate, and may exert allelopathic effects on microorganisms and plants (Herbert and Bertsch 1995; Zsolnay 2003).

Materials and Reagents

1 1.5 mL Eppendorf tubes.

2 Spectrophotometer.

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3 1-cm path length quartz or optical glass cuvettes.

4 Microcentrifuge.

5 Folin–Ciocalteu's phenol reagent (available from SIGMA); store in the dark.

6 Saturated Na_2CO_3 solution: dissolve 216 g in 1 L of deionized water.

7 Stock standard solution: 2-hydroxybenzoic acid (analytical grade, 100 mg L^{-1}) deionized water.

8 Working standards: prepare solutions of 2.5, 5, 10, 20, 30, and 40 mg L^{-1} 2-hydroxybenzoic acid by dilution of the stock solution.

Procedure and Calculations

1 Add 0.7 mL of DOM sample, standard, or blank into a 1.5 mL Eppendorf tube.

2 Add 50 μL of Folin–Ciocalteu's reagent.

3 Mix and let stand for 3 min at room temperature.

4 Add 100 μL of the saturated Na_2CO_3 solution.

5 Add 150 μL of deionized water, mix well, and let stand for 10 to 20 min at room temperature.

6 Blue color should develop if phenols are present; blanks should go colorless. If a precipitate is formed, centrifuge for 2 to 3 min (2,000 *g*) and read absorbance immediately.

7 Transfer a sufficient amount of the sample or standard to a glass cuvette and read absorbance at 725 nm against blank.

8 Prepare calibration curve and calculate phenol concentration in mg L^{-1} 2-hydroxybenzoic acid equivalent.

Comments

1 This method can be used for all kinds of aqueous soil extracts, including alkaline soil extracts (Morita, 1980).

2 The method precisely detects phenol concentrations as small as 1 mg L^{-1} 2-hydroxybenzoic acid equivalent.

3 Folin–Ciocalteu's reagent reacts with various phenol monomers and polyphenolic substances. The reaction products vary in their color yields and adsorption maxima. Thus, the method does not accurately measure the total phenol concentration but allows for qualitative comparison of samples. The limitation of the proposed method in estimating phenol concentration has been discussed in detail

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by Box (1983) and Ohno and Paul (1998). Rough estimates of monomeric and polymeric phenols can be achieved by precipitating polyphenols with casein and analyzing the supernatant for monomeric phenol concentration. Polyphenol concentration is estimated as the difference between total phenol concentration and monomeric phenol concentration in the precipitated sample (Kuiters and Denneman 1987). Phenols other than 2-hydroxybenzoic acid (e.g., tannins, vanillic acid, gallic acid) can be used as standards. High concentrations of Fe^{2+}, Mn^{2+}, or S^{2-} in samples interfere with the method, likely resulting in overestimation of phenol concentration.

48.3.5 Hexoses

Hexoses in soil DOM are an energy source to soil microbes and therefore may be used as an indicator of DOM biodegradability (DeLuca and Keeney 1993). Apart from glucose, most hexoses in soil are of microbial origin (see Chapter 50).

Materials and Reagents

1. 10 mL glass test tubes.
2. Vortex mixer.
3. Spectrophotometer.
4. 1-cm path length cuvettes (optical glass or plastic is acceptable).
5. Anthrone–sulfuric acid reagent: dissolve 0.2 g of anthrone (analytical grade) in 100 mL of concentrated (96%–98% v/v) H_2SO_4 (analytical grade), prepare always fresh for the day but let it stand for ca. 1 h at room temperature before use.
6. Stock standard: glucose (analytical grade), 100 mg L^{-1}, in deionized water.
7. Working standards: prepare solutions of 2.5, 5, 10, 20, 30, and 50 mg L^{-1} glucose by dilution from stock standard.

Procedure and Calculations

1. Add 1 mL of DOM sample, standard, or blank to a test tube.
2. Add 2 mL of anthrone–sulfuric acid reagent (beware, the solution heats up).
3. Vortex and let stand for 15 min at room temperature.
4. Transfer a sufficient amount of the anthrone-treated sample or standard to a cuvette and read absorbance at 625 nm against the blank.
5. Prepare calibration curve and calculate hexose concentration in mg L^{-1} glucose equivalent.

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Comments

1. This method is a variant of a procedure originally proposed for measurement of carbohydrates in soil hydrolysates (Brink et al. 1960).

2. Typically, the sulfuric acid added with the anthrone reagent is sufficient to hydrolyze the dissolved polysaccharides.

3. The method seems to be robust against interferences from Fe^{2+}, Mn^{2+}, NO_3^-, and Cl^- ions. However careful control, such as a spike-recovery experiment, along with meticulous analytical procedures seem important in obtaining reliable results (Grandy et al. 2000).

4. Glass test tubes should be carefully rinsed with ultrapure water before use to avoid contamination with hexoses. As with all tests using a single compound for calibration, the method tends to overestimate the hexose concentration. For a more detailed analysis of soil hexoses, chromatographic procedures are described in Chapter 50.

48.3.6 Pentoses

Pentoses are an energy source to soil microbes and measuring pentoses along with hexoses provides a measure of the total abundance of carbohydrates in DOM. Pentoses in soil are mainly of plant origin (see Chapter 50). Therefore, the ratio of pentoses-to-hexoses may provide information about the relative abundance of plant-derived carbohydrates in DOM.

Materials and Reagents

1. 10 mL glass tubes equipped with Teflon-lined screw caps.

2. Water bath: operating at 95°C.

3. Spectrophotometer.

4. 1-cm path length cuvettes (optical glass or plastic is acceptable).

5. Iron chloride reagent: dissolve 0.1 g of $FeCl_3$ (analytical grade) in 100 mL of concentrated (32% v/v) HCl (analytical grade).

6. 95% ethanol (analytical grade).

7. Orcinol reagent: dissolve 1 g of orcinol ($CH_3C_6H_3$-1,3-$(OH)_2$; 3,5-dihydroxy-toluene) in 100 mL of 95% ethanol (analytical grade).

8. Stock standard: ribose (analytical grade), 100 mg L^{-1}, in deionized water.

9. Working standards: prepare solutions of 2.5, 5, 10, 20, 30, and 50 mg L^{-1} ribose by dilution from stock standard.

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Procedure and Calculations

1. Add 1 mL of DOM sample, standard, or blank into a test tube.
2. Add 1 mL of iron chloride reagent and 1 mL of orcinol reagent, cap the tubes.
3. Keep in the water bath at 95°C for 20 min.
4. Cool on ice for 5 min.
5. Add 2 mL of 95% ethanol, mix.
6. Transfer a sufficient amount of the treated sample or standard to a cuvette and read absorbance at 660 nm against blank.
7. Prepare calibration curve and calculate pentose concentration in mg L^{-1} ribose equivalent.

Comments

1. This method is adapted from a procedure developed for pentoses released from biogenic tissues (Mejbaum 1939). Recently it has been applied to aqueous samples and proved to give reasonable and reliable estimates of total pentose content in DOM (Kawahigashi et al. 2003).
2. As with all tests using a single compound for calibration, the method tends to overestimate the pentose concentration. Test tubes should be rinsed thoroughly with ultrapure water before use to avoid contamination with pentoses. For a more detailed analysis of soil pentoses, chromatographic procedures are described in Chapter 50.

48.3.7 Amino Acids

Free amino acids are found at low concentrations in DOM and thought to be a significant source of N to plants in natural ecosystems and to soil microbes (Jones and Kjelland 2002).

Materials and Reagents

1. 10 mL glass tubes equipped with Teflon-lined screw caps.
2. Water bath: operating at 95°C.
3. Water bath: at room temperature.
4. Spectrophotometer.
5. 1-cm path length cuvettes (optical glass or plastic is acceptable).

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6 Acetate buffer (pH 5.5): dissolve 54 g of Na acetate trihydrate (analytical grade) in 40 mL of deionized water, add 10 mL of glacial acetic acid, adjust pH to 5.5 with NaOH.

7 Ninhydrin reagent: dissolve 2 g of ninhydrin (analytical grade) and 0.3 g of hidrindantin in 75 mL of 2-hydroxy ethanol (analytical grade), purge the solution with N_2 for 30 min, then add 25 mL of acetate buffer (pH 5.5). Prepare solution always fresh and avoid contact with air as much as possible.

8 Dilutant: mix equal amounts of 95% ethanol (analytical grade) and deionized water.

9 Stock standard: leucine (analytical grade), 1000 μmol L^{-1}, in deionized water.

10 Working standards: prepare solutions of 20, 40, 60, 80, and 100 μmol L^{-1} leucine by dilution from stock standard.

Procedure and Calculations

1 Add 2 mL of DOM sample, standard, or blank into a test tube.

2 Slowly add 1.25 mL of the ninhydrin reagent, cap the tubes.

3 Keep in the water bath at 95°C for 25 min.

4 Cool to room temperature in another water bath.

5 Add 4.5 mL of dilutant, mix.

6 Transfer a sufficient amount of the treated sample or standard to a cuvette and read absorbance at 570 nm against blank.

7 Prepare calibration curve and calculate amino acids concentration in μmol L^{-1} leucine equivalent.

Comments

1 This procedure is a variant of the original method by Moore and Stein (1948).

2 The most critical point in the analytical procedure is to keep the ninhydrin reagent from reacting with oxygen. An alternative spectrofluorometric method for the sensitive determination of amino acids was recently proposed by Jones et al. (2002) and relies on the reaction of free amino acids with *o*-phthaldialdehyde and beta-mercaptoethanol.

48.3.8 Proteins

Proteins may be important contributors to dissolved organic N and the source for replenishment of the free amino acids pool in DOM. They therefore have a potential influence on microbial growth in soil.

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Materials and Reagents

1. Spectrophotometer.
2. 1-cm path length cuvettes (optical glass or plastic is acceptable).
3. Bradford protein reagent (available from SIGMA), store refrigerated.
4. Stock standard: bovine serum albumin (BSA), dissolve 100 mg BSA L^{-1} deionized water. Prepare fresh as it does not preserve well even when frozen.
5. Working standards: prepare solutions of 2.5, 5, 10, 15, 20, and 25 mg L^{-1} BSA by dilution from stock standard.

Procedure and Calculations

1. Add 0.5 mL of Bradford protein reagent into a spectrophotometer cuvette.
2. Add 0.5 mL of DOM sample, standard, or blank.
3. Mix well and let stand for 5 min at room temperature.
4. Read absorbance at 620 nm against blank.
5. Prepare calibration curve and calculate protein concentration in mg L^{-1} BSA equivalent.

Comments

Determination of proteins in aqueous samples is recommended only when samples are fresh. Upon storage, protein concentration seems to decrease rapidly. The method allows for detection of protein concentrations as small as 1 mg L^{-1} BSA equivalent.

48.3.9 Assessment of Biodegradability

Dissolved organic matter represents a potential source of energy and nutrients (especially N and P) to the soil microflora. Microbial consumption of DOM can regulate the production of greenhouse gases such as CH_4 and N_2O both by reducing the O_2 content of soils and by providing the electrons required for methanogenesis and denitrification (Yavitt 1997; Zsolnay 1997; Lu et al. 2000). Furthermore, knowledge about the biodegradability of DOM is also crucial to assessing its contribution to soil organic matter buildup (Kalbitz et al. 2003). The biodegradation of DOM is defined by the metabolic breakdown of these organic compounds by soil microorganisms; it is assessed either by the disappearance rate of DOM, the consumption rate of O_2, or the evolution rate of CO_2 during incubation assays (Marschner and Kalbitz 2003).

Materials and Reagents

1. Teflon, glass, or polypropylene flasks (50 mL) for the incubation
2. Soil inoculum (obtained by shaking a fresh soil sample with 5 m*M* $CaCl_2$ solution for 30 min in a 50 mL polypropylene tube, and filtering at 5 μm)

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3 1 g L^{-1} NH_4NO_3 and 1 g L^{-1} K_2HPO_4 aqueous solutions

4 DOM samples

5 Glass vacuum filter holder or stainless steel pressure filtration unit

6 0.4 μm polycarbonate filter that fits the filter holder

7 5 μm polycarbonate filter that fits the filter holder to filter the inoculum

8 125 mL Erlenmeyer flask to support the filter holder (with sidearm if a vacuum filter unit is used)

9 Vacuum pump or other vacuum/pressure system

Procedures and Calculations

1 Transfer/pipette 15 mL of DOM sample (diluted to a C concentration of 10 to 30 mg C L^{-1}) in a Teflon, glass, or polypropylene flask and add 3 mL of each of the NH_4NO_3 and K_2HPO_4 solutions. Prepare all tested DOM samples at least in triplicates.

2 Prepare the microbial inoculum and filter through a 5 μm filter to remove large particles and grazers.

3 Add 150 μL of the inoculum and incubate the capped flasks in the dark at room temperature for 7 days.

4 Two different controls should be prepared: ultrapure water with nutrients and the inoculum can be used to quantify the DOC coming from the inoculum. A second control using a glucose solution instead of the DOM sample is useful to verify the functioning of the inoculum; in this test more than 50% of the glucose-C should be degraded within 7 days.

5 Determine the organic C concentration (see Section 48.3.1) of the filtered (0.4 μm) samples before and after the 7-day incubation. The difference represents the amount of biodegradable DOC.

Comments

1 Since the purpose of this method is to assess readily biodegradable DOM, longer incubation periods will result in larger net removal of organic C from solution (or greater mineralization) and thus give higher estimates of biodegradability (McDowell et al. 2006).

2 Addition of nutrients is recommended because nutrient deficiency may limit the degradation of DOM and result in underestimation of its true biodegradability (McDowell et al. 2006).

3 Comparison of different methods suggests that adding soil inoculum derived from the same soil where DOM samples are obtained is not necessary (McDowell et al. 2006).

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4 Size of the incubation vessel should be chosen to avoid oxygen deficiency; the ratio of liquid to headspace volume during the incubation trial should be about 1 or lower.

5 In addition to short-term batch incubations for measurement of biodegradable DOC, McDowell et al. (2006) suggested 42-day incubations with regular monitoring of headspace CO_2 concentrations, conducted at room temperature with an inoculum and nutrients added. Regular monitoring of headspace CO_2 concentration offers additional information by providing biodegradability estimates of both the labile and refractory portions of DOC, which is useful for modeling the fate of DOC in soils.

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Chapter 49
Soil Microbial Biomass C, N, P, and S

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49.1 INTRODUCTION

Soil microbial biomass measurements have been used in studies of soil organic matter dynamics and nutrient cycling in a variety of terrestrial ecosystems. They provide a measure of the quantity of living microbial biomass present in the soil, and in arable soils account for ~1%–5% of the total soil organic matter (Jenkinson 1988; Smith and Paul 1990). Measurements of the carbon (C), nitrogen (N), phosphorus (P), and sulfur (S) contained in the soil microbial biomass provide a basis for studies of the formation and turnover of soil organic matter, as the microbial biomass is one of the key definable fractions (Brookes et al. 1990). The data can be used for assessing changes in soil organic matter caused by soil management (Powlson et al. 1987) and tillage practices (Spedding et al. 2004), for assessing the impact of management on soil strength and porosity, soil structure and aggregate stability (Hernández-Hernández and López-Hernández 2002), and for assessing soil N fertility status (Elliot et al. 1996).

Because it is such a sensitive indicator of changing soil conditions, the soil microbial biomass serves as an ''early warning'' of effects of stresses on the soil ecosystem, long before they are detectable by other measurements (Barajas Aceves et al. 1999). Soil microbial biomass measurements have been used for determining the effects of environmental contaminants like heavy metals (Renella at al. 2004; Barajas Aceves 2005), pesticides (Harden et al. 1993), and antibiotics (Castro et al. 2002) on the soil ecosystem, and to

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monitor bioremediation of oil-contaminated soils (Plante and Voroney 1998). Linked measurements such as the CO_2 evolved per unit soil microbial biomass, i.e., biomass specific respiration rate, and the microbial biomass as a percentage of soil organic C are useful practical indicators (Barajas Aceves et al. 1999).

Jenkinson (1988) and Smith and Paul (1990), and more recently Carter et al. (1999), have reviewed the significance of soil microbial biomass measurements. There have been several reviews of methods for measurement of the microbial biomass (Horwath and Paul 1994; Joergensen and Brookes 2005).

A wide range of techniques has been used to, directly or indirectly, quantify the size of the microbial biomass in soil. These include direct microscopic counts of soil organisms and conversion to biomass from estimates of organism biovolume, density, solids content, and carbon content (van Veen and Paul 1979); conversion of soil adenosine 5′-triphosphate (ATP) concentrations to microbial biomass (Contin et al. 2001; Dyckmans et al. 2003); arginine mineralization (Lin and Brookes 1999); anthrone reactive C (Badalucco et al. 1992); ninhydrin-reactive N (Amato and Ladd 1988; Mondini et al. 2002); and the substrate-induced respiratory response (Anderson and Domsch 1978; Graham and Haynes 2005). Microbial biomass determinations were first based on measuring the CO_2 produced as a result of chloroform ($CHCl_3$) fumigation during a subsequent 10-day incubation (Jenkinson 1966). Exposing the soil to $CHCl_3$ vapors kills all of the soil organisms by solubilizing the lipids in the cell membrane, releasing the cellular constituents into the soil. Removal of the fumigant and incubation of the fumigated soil results in a ''flush'' of decomposition by the recolonizing population, compared with an unfumigated soil, due to mineralization of these cellular constituents. This flush of CO_2 was shown to be directly proportional to the amount of microbial biomass C. The original fumigation–incubation method to measure soil biomass C (Jenkinson and Powlson 1976) and N (Voroney and Paul 1984) is considered the reference procedure against which other methods are calibrated (Ross 1990). However, it has largely been replaced by the fumigation–extraction method, where the biomass constituents released by $CHCl_3$ fumigation are extracted and analyzed directly to reduce the time required to complete the technique. In this chapter, the fumigation–extraction methods for measurements of soil microbial biomass C, N, P, and S are described.

49.2 CHLOROFORM FUMIGATION–EXTRACTION METHOD

Estimation of soil microbial C, N, P, and S by fumigation–extraction methods has several advantages over the fumigation–incubation method. Besides being rapid, the fumigation–extraction method is applicable to soils of low pH (Couteaux and Henkinet 1990), and to those with recent additions of organic substrates (Vance et al. 1987). Also it avoids the requirement for microbial mineralization of the fumigant-killed biomass during a 10-day incubation period under controlled conditions (Vance et al. 1987; Amato and Ladd 1988; Gallardo and Schlesinger 1990). Therefore, the method can be applied to soils of low initial water content, provided the soils are wetted to between -5 and -10 kPa moisture potential during the fumigation stage (Sparling et al. 1990), and to waterlogged soils or paddy soils (Inubushi et al. 1991). Diaz-Raviña et al. (1992) have used the method in soils heated to 600°C. However, the main advantage is that fumigation–extraction permits microbial biomass measurements in soils with recently added and freshly decomposing substrates (Ocio and Brookes 1990a), and it is ideal for use in conjunction with isotopically labeled substrates (Wu and Brookes 2005).

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49.3 MICROBIAL BIOMASS C AND N

Microbial biomass C and N are calculated from the difference between the amount of total C and N extracted from fresh soil fumigated with $CHCl_3$ and the amount extracted from the unfumigated control soil. The total C and N released by $CHCl_3$ fumigation and present in the unfumigated soil are extracted with 0.5 *M* K_2SO_4 and subsequently analyzed (Sparling and West 1988a). The microbial biomass C and N are calculated using an equation relating the increased release of C and N as a result of $CHCl_3$ fumigation and a factor representing the fraction of biomass C and N extracted by K_2SO_4.

49.3.1 Materials and Reagents

Most of the reagents used, except where noted, are readily obtainable and are certified analytical grade. Deionized water (Type 1) should be used throughout for the preparation of the reagent solutions.

1. Ethanol-free $CHCl_3$. See Comment 1 in Section 49.6 for details outlining the purification of reagent grade $CHCl_3$.

2. Large desiccator. The desiccator should be inert to $CHCl_3$ vapor, be of a dry-seal type, and be able to withstand a high vacuum without implosion; a thick-walled glass vacuum desiccator is suitable. A large desiccator may hold about 25 samples simultaneously.

3. 0.5 *M* K_2SO_4 extraction solution. To prepare 10 L of extractant, slowly add 871.3 g K_2SO_4 (reagent grade) to about 9.5 L water. Stir the solution on a magnetic stirrer until the K_2SO_4 has dissolved completely (~2 h); adjust the final volume to 10 L with water after the solution has reached room temperature.

4. Miscellaneous materials and reagents:

   a. Fumehood

   b. Boiling chips

   c. 100 mL glass containers with lids

   d. Aluminum-weighing containers for the determination of soil water content

   e. Glass fiber filter papers (Whatman® GF 934-AH)

   f. 50 mL vials per assay for filtrate

49.3.2 Soil Preparation

1. For the standard method, measurements are usually made on sieved soil (<2 mm) that has been preincubated, at 25°C and 40%–50% water holding capacity for several days, to permit soil metabolism and moisture to stabilize

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(e.g., Vance et al. 1987). If this is not practical, the soil may be divided into small (<1 cm) pieces and mixed thoroughly prior to fumigation (Ocio and Brookes 1990b), however, it is necessary to use larger soil weights (ca 250 g) than in the standard method. In soils containing large amounts of fresh plant residues and living roots, these must be removed by hand picking or sieving (Mueller et al. 1992).

2 Weigh out three portions of the moist soil, ~15 g, into a weighing container for soil water content determination. Dry soil in the weighing container in an oven at 105°C for at least 24 h or until a constant oven-dry weight is achieved. Cool in a desiccator, reweigh, and determine the water content of the soil sample. All microbial biomass results should be expressed on an oven-dry weight basis.

3 Weigh out six portions of moist soil, 25–50 g each (standard method), into 100 mL glass bottles. This provides (i) three replicates of the soil to be fumigated with $CHCl_3$ vapor for 24 h and then extracted and (ii) three replicates of the unfumigated soil that are extracted immediately.

49.3.3 Fumigation Treatment

Caution: Procedures releasing $CHCl_3$ fumes should be conducted in a fumehood

1 Desiccators should be lined with freshly moistened paper towels. For the fumigation treatment, place the glass sample bottles containing the soil into a desiccator together with a 100 mL beaker containing 50 mL $CHCl_3$ and a few boiling chips. Seal and evacuate the desiccator, taking care to vent the fumes released by the vacuum pump until the $CHCl_3$ boils vigorously and continue evacuating for 1–2 min. Seal the desiccator under vacuum and place it in the dark at room temperature (20°C–25°C) for 24 h.

2 After the 24 h fumigation, release the vacuum, open the desiccator, and remove the beaker of $CHCl_3$ and moistened paper towels. (The waste $CHCl_3$ should be kept in a sealed bottle and disposed as a hazardous waste; the paper towels should be placed in sealed bags and disposed in the regular waste stream.)

3 Remove residual $CHCl_3$ vapor from the soil samples by repeated evacuations, usually three to six times for ~5 min using a water aspirator pump, followed by a two-stage rotary oil pump capable of drawing a vacuum of 10^{-5} kPa, 15–20 min evacuation.

49.3.4 Extraction of Microbial Biomass C and N

1 Add 0.5 *M* K_2SO_4 to the bottles containing the unfumigated control and fumigated samples using the equivalent oven-dry soil weight (g):extractant volume (mL) ratio of 1:2 to 1:5 (Joergensen and Olfs 1998).

2 Cap the jars and place on a shaker (oscillating or rotary) for 1 h. After shaking, filter the soil suspension through Whatman GF 934-AH filter paper. Measurements of organic C and total N can require from 5 to 20 mL of extract depending on the methods of analysis used. Cap and store the filtrate at 4°C for not more than 2 to 3 days, otherwise, freeze until ready for analysis. Upon thawing of frozen K_2SO_4

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soil extracts, a white precipitate of $CaSO_4$ occurs in near-neutral or alkaline soils. However, this causes no analytical problems in either method and may be safely ignored (Joergensen and Olfs 1998).

49.3.5 Determination of Extracted C and N

1 Organic C dissolved in the soil extracts can be measured using automated combustion or wet oxidation procedures. However, the preferred method to quantify C in the extracts utilizes high-temperature total C analyzers. These oxidize C in the extracts to CO_2 after removing inorganic C from the sample and determine the concentration of CO_2 generated with an infrared detector. Quantification of C in the extracts can also be determined by UV-catalyzed wet oxidation followed by detection of the CO_2 generated with an infrared detector. Alternatively, C in the extracts can be determined colorimetrically. The determination involves a pretreatment to remove inorganic C by entraining the acidified sample with a high velocity stream of N- or C-free air to purge carbonate-derived CO_2. The sample is then mixed with 0.5 *M* H_2SO_4 and 4% (w/v) potassium persulfate and subjected to UV radiation. The resultant CO_2 generated from the organic C present in the sample is then dialyzed through a silicone rubber membrane and reacted with a weakly buffered phenolphthalein indicator. The decrease in the intensity of the color of the indicator measured at 550 nm is proportional to the organic C content. Standard solutions ranging in concentration from 0 to 150 μg C mL^{-1} are prepared by dilution of a stock potassium biphthalate solution containing 1000 mg C.

2 Organic N in the soil extracts can be determined with the method outlined by Cabrera and Beare (1993). In this procedure, dissolved organic N and NH_4^+ are oxidized to NO_3^- by persulfate. The oxidation can be conveniently run in an autoclave with screw-cap tubes and yields a nontoxic salt solution in which the NO_3^- concentration can be determined using a colorimetric technique involving the Griess–Ilosvay reaction after Cd reduction. Details of the oxidation procedure are provided in Chapter 48 (Section 48.3.2), and details of the measurement of the NO_3^- and NO_2^- concentrations in the extract are provided in Chapter 6.

49.3.6 Calculation of Microbial Biomass C and N

1 Soil water content, expressed on an oven-dry basis (WS):

$$\text{WS (\%)} = [[\text{soil wet weight (g)} - \text{soil dry weight (g)}]/\text{soil dry weight (g)}] \times 100 \quad (49.1)$$

2 Weight of soil sample (oven-dry weight equivalent) taken for microbial biomass measurements (MS):

$$\text{MS (g)} = \text{soil wet weight (g)} \times 100/(100 + \text{WS (\%)}) \quad (49.2)$$

3 Total volume of solution in the extracted soil (VS):

$$\text{VS (mL)} = [(\text{soil wet weight (g)} - \text{soil dry weight (g)})/1\text{g mL}^{-1}] + \text{extractant volume (mL)} \quad (49.3)$$

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4 Total weight of extractable C in the fumigated (C_F) and unfumigated (C_{UF}) soil samples:

$$C_F,\ C_{UF}\ (\mu g\ g^{-1}\ soil) = \text{organic C}\ (\mu g/mL) \times [VS\ (mL)/MS\ (g)] \quad (49.4)$$

5 Total weight of extractable N in the fumigated (N_F) and unfumigated (N_{UF}) soil samples:

$$N_F,\ N_{UF}\ (\mu g\ g^{-1} soil) = \text{total N}\ (\mu g/mL) \times [VS\ (mL)/MS\ (g)] \quad (49.5)$$

6 Microbial biomass C in the soil (MB-C):

$$\text{MB-C}\ (\mu g\ g^{-1}\ soil) = (C_F - C_{UF})/k_{EC} \quad (49.6)$$

where $k_{EC} = 0.35$ and represents the efficiency of extraction of microbial biomass C. Values for k_{EC} range from 0.25 to 0.45 (Wu et al. 1990; Joergensen 1996).

7 Microbial biomass N in the soil (MB-N):

$$\text{MB-N}\ (\mu g\ g^{-1}\ soil) = (N_F - N_{UF})/k_{EN} \quad (49.7)$$

where $k_{EN} = 0.5$ and represents the efficiency of extraction of microbial biomass N. Values for k_{EN} range from 0.18 to 0.54 (Joergensen and Mueller 1996).

49.4 MICROBIAL BIOMASS P

Microbial biomass P is calculated from the difference between amounts of inorganic P (P_i) extracted from fresh soil fumigated with $CHCl_3$ and the amount extracted from unfumigated soil (Brookes et al. 1982; Hedley and Stewart 1982). Most of the P released is inorganic (i.e., orthophosphate-P_i). The extractant is 0.5 *M* $NaHCO_3$ (pH 8.5). For acidic soils, Wu et al. (2000) recommended the use of Bray-1 reagent (0.03 *M* NH_4F–0.025 *M* HCl), while Kouno et al. (1995) demonstrated that an anion exchange membrane technique could be used for biomass P measurements in acidic soils and soils of high P retention capacity. Some of the P_i released following fumigation is sorbed to soil colloids, so it is necessary to determine the recovery efficiency for each soil. Recovery efficiency is obtained by spiking a known quantity of P_i to the soil during extraction and measurement of the P_i recovered. The microbial biomass P is calculated by an equation relating the increased release of P_i as a result of $CHCl_3$ fumigation and the fraction of killed biomass P_i extracted by $NaHCO_3$, corrected for the recovery efficiency of the procedure.

49.4.1 Materials and Reagents

1 Ethanol-free $CHCl_3$. See Comment 1 in Section 49.6 for details outlining the purification of reagent grade $CHCl_3$.

2 Large desiccator. The desiccator should be inert to $CHCl_3$ vapor, be of a dry-seal type, and be able to withstand a high vacuum without implosion; a thick-walled glass vacuum desiccator is suitable. A large desiccator may hold about 25 samples simultaneously.

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3 0.5 *M* $NaHCO_3$ at pH 8.5 extraction solution (Olsen et al. 1954). To prepare 10 L of the extractant, weigh 420 g $NaHCO_3$ into about 9 L water, add 7.2 g NaOH pellets, adjust the pH to 8.5 with 10 *M* NaOH (or conc. H_2SO_4) if necessary, and then adjust to 10 L with water. Extreme accuracy of reagent concentrations is not required, however, the precise pH of the extractant is critical.

4 250 μg P_i mL^{-1} spiking solution. To prepare 1 L of the spiking solution, dissolve 1.098 g KH_2PO_4 in 900 mL water in a 1 L volumetric flask and bring to volume.

5 Miscellaneous materials and reagents:

a. Fumehood

b. Boiling chips

c. 250 mL glass conical flasks with stoppers or caps

d. Aluminum-weighing containers for determination of soil water content

e. Whatman 42 filter paper

f. 50 mL vials per assay for filtrate

49.4.2 Soil Preparation

1 Sieve and mix soil as described in Section 49.3.2.

2 Weigh and dry soil to determine soil moisture content as described in Section 49.3.2.

3 Weigh out nine portions of moist soil, each containing ~10 g soil (oven-dry basis), into 250 mL conical flasks. This provides (i) three replicates of the soil to be fumigated with $CHCl_3$ vapor for 24 h and then extracted, (ii) three replicates of the unfumigated soil to be kept for 24 h in a sealed desiccator containing water and soda-lime and then extracted, and (iii) three replicates of the unfumigated soil to be kept for 24 h in a sealed desiccator containing water and soda-lime and then extracted with the extractant spiked with P_i.

49.4.3 Fumigation Treatment

1 Fumigation procedures are described in Section 49.3.3.

49.4.4 Extraction of Microbial Biomass P

1 Add 200 mL of the extractant +1 mL water to each of the flasks containing the unfumigated control and fumigated samples, using the equivalent oven-dry soil weight (g):extractant volume (mL) ratio of 1:2 to 1:5.

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2 Add 200 mL of the extractant +1 mL of spiking solution to each of the three replicate flasks containing the unfumigated samples to be extracted with spiked extractant using the same soil:extractant ratio as described above.

3 Set of three extractant blanks is included for each full run. The mixtures are shaken at 20°C for 30 min on an orbital shaker (150 rev min^{-1}), then filtered through Whatman 42 filter paper into acid-washed plastic bottles.

49.4.5 Determination of Extracted P

The increase in P_i due to $CHCl_3$ fumigation is used to estimate microbial biomass P. Determination of the P_i present in the soil extracts is measured by the method of Murphy and Riley (1962). Briefly, ammonium molybdate and antimony potassium tartrate react in an acidic environment with phosphate to form an antimony-phospho-molybdate complex. Ascorbic acid is then used to reduce this complex to an intensely blue-colored complex. The absorbance determined at a wavelength of 712 nm is proportional to the phosphate concentration present in the sample over a defined range. A detailed description of the procedure is provided in Chapter 25 (Section 25.4.5).

49.4.6 Calculation of Microbial Biomass P

1 Soil water content (WS) is determined by Equation 49.1.

2 Mass of dry soil (MS) is determined by Equation 49.2.

3 Total volume of solution in the extracted soil (VS):

$$\begin{aligned} \text{VS (mL)} = {} & [\text{soil wet weight (g)} - \text{soil dry weight (g)}/1\ \text{g mL}^{-1}] \\ & + \text{extractant volume (mL)} + 1\ \text{mL (either water or } P_i \text{ spike)} \end{aligned} \tag{49.8}$$

4 Total weight of extractable P_i in the fumigated (F) and unfumigated (UF) soil samples:

$$P_F,\ P_{UF}\ (\mu g\ g^{-1}\ \text{soil}) = P_i\ (\mu g/mL) \times [\text{VS (mL)}/\text{MS (g)}] \tag{49.9}$$

5 Total weight of extractable P_i in the P_i spiked soil samples:

$$P_i \text{ spiked soil } (\mu g\ g^{-1}\ \text{soil}) = P_i \text{ spiked soil } (\mu g/mL) \times [\text{VS (mL)}/\text{MS (g)}] \tag{49.10}$$

6
$$\text{MB-P } (\mu g\ g^{-1}\ \text{soil}) = [(P_F - P_{UF})/k_{EP}] \times (100/R) \tag{49.11}$$

where $k_{EP} = 0.40$ and represents the efficiency of extraction of microbial biomass P, and $R = 100\ [(P_i \text{ spiked soil} - \text{soil } P_{UF})/P_i \text{ spike}]$, and is the percent recovery of the P_i spike, and P_i spike $= 250\ \mu g\ P_i$.

49.5 MICROBIAL BIOMASS S

Microbial biomass S is calculated from the difference between the amount of total S extracted from fresh soil fumigated with $CHCl_3$ and the amount extracted from unfumigated soil (Sagger et al. 1981). Both organic and inorganic forms of S are extracted with $CaCl_2$

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during the procedure and analysis requires that organic S is converted to inorganic S to determine the total S extracted. The microbial biomass S is calculated by an equation relating the increased release of S as a result of $CHCl_3$ fumigation and the fraction of killed biomass S extracted by $CaCl_2$.

49.5.1 Materials and Reagents

1. Ethanol-free $CHCl_3$. See Comment 1 in Section 49.6 for details outlining the purification of reagent grade $CHCl_3$.

2. Large desiccator. The desiccator should be inert to $CHCl_3$ vapor, be of a dry-seal type, and be able to withstand a high vacuum without implosion; a thick-walled glass vacuum desiccator is suitable. A large desiccator may hold about 25 samples simultaneously.

3. 0.10 *M* $CaCl_2$ extraction solution (Saggar et al. 1981). To prepare 10 L of extracting solution, add 14.7 g $CaCl_2 \cdot 2H_2O$ to about 9.5 L water. Place the solution container on a magnetic stirrer and stir until the $CaCl_2 \cdot 2H_2O$ has dissolved completely and then adjust the final volume to 10 L with water.

4. Miscellaneous materials and reagents:

   a. Fumehood

   b. Boiling chips

   c. 100 mL glass bottles with lids

   d. Aluminum-weighing containers for determination of soil water content

   e. 0.45 μm Millipore filter (Millipore Corp., Bedford, MA)

   f. 50 mL vials per assay for filtrate

49.5.2 Soil Preparation

1. Sieve and mix soil as described in Section 49.3.2.

2. Weigh and dry soil to determine soil moisture content as described in Section 49.3.2.

3. Weigh out six portions of moist soil, 15–50 g each, into 100 mL glass bottles. This provides (i) three replicates of the soil to be fumigated with $CHCl_3$ vapor for 24 h and then extracted and (ii) three replicates of the unfumigated soil to be extracted immediately.

49.5.3 Fumigation Treatment

1. Fumigation procedures are described in Section 49.3.3.

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49.5.4 Extraction of Microbial Biomass S

1 Add 0.01 *M* $CaCl_2$ to the bottles containing the unfumigated control and fumigated samples, using the equivalent oven-dry soil weight (g):extractant volume (mL) ratio of 1:2 to 1:5 (Wu et al. 1994).

2 Cap the jars and place on a shaker (oscillating or rotary) for 1 h. After shaking, pass the soil suspension through 0.45 μm Millipore filters. Cap and store the filtrate at 4°C for not more than 2 to 3 days, otherwise freeze until ready for analysis.

49.5.5 Determination of Extracted S

The increase in total S due to $CHCl_3$ fumigation is used to estimate microbial biomass S. Determination of the total S present in the soil extracts involves two steps: (i) conversion of the various S compounds in the extract to one form and (ii) determination of the concentration of the S present. The recommended procedure is an alkaline digestion of the organic S to sulfate followed by reduction to sulfide with HI (Tabatabai and Bremner 1970). The determination of sulfide is then completed colorimetrically. Detailed descriptions of the procedures to convert organic S to sulfate and for the determination of sulfate by reduction to sulfide are given in Chapter 23.

49.5.6 Calculation of Microbial Biomass S

1 Soil water content (WS) is determined by Equation 49.1.

2 The mass of dry soil (MS) is determined by Equation 49.2.

3 Total volume of solution in the extracted soil (VS) is determined by Equation 49.3.

4 Total weight of extractable S in the fumigated (F) and unfumigated (UF) soil samples:

$$S_F,\ S_{UF}\ (\mu g\ g^{-1}\text{soil}) = \text{Total S}\ (\mu g/mL) \times [\text{VS}\ (mL)/\text{MS}\ (g)] \tag{49.12}$$

5 Microbial biomass S in the soil (MB-S):

$$\text{MB-S}\ (\mu g\ g^{-1}\text{soil}) = (S_F - S_{UF})/k_{ES} \tag{49.13}$$

where $k_{ES} = 0.35$ and represents the efficiency of extraction of microbial biomass S. Values for k_{ES} range from 0.30 to 0.35 (Wu et al. 1994).

49.6 COMMENTS

1 Reagent grade $CHCl_3$ is normally stabilized with ethanol (~1% v/v). Ethanol-free $CHCl_3$ must be used to measure microbial biomass C because ethanol cannot be completely removed from the soil after fumigation. Ethanol-stabilized $CHCl_3$ can be used if only microbial biomass N is measured (DeLuca and Keeney 1993). The purification procedure consists of double distillation of $CHCl_3$ at 55°C, followed by washing three times with 18 *M* H_2SO_4 and then five times with deionized

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water (Jenkinson and Powlson 1976). Purified $CHCl_3$ is kept in a brown bottle containing anhydrous K_2CO_3 and redistilled. The purified $CHCl_3$ can be stored over anhydrous K_2CO_3 in the dark for up to 3 weeks. Though two to three times more costly, the use of amylene (2-methyl 2-butene) stabilized $CHCl_3$ (HPLC grade) simplifies the procedure, greatly reducing exposure to hazardous $CHCl_3$ fumes (Jenkinson and Powlson 1976), and gives identical results to freshly distilled $CHCl_3$ (Mueller et al. 1992).

2 Weight of soil analyzed for microbial biomass C and N can range from 200 mg (Daniel and Anderson 1992) to 200 g (Ocio and Brookes 1990b). However, to minimize sampling error and increase the precision of the determinations, it is recommended that determinations be completed on a minimum sample size equivalent to 25 g of oven-dried soil.

3 Soils, which do not readily disperse in the extracting solution, should be homogenized before shaking (Winter et al. 1994). Soil samples can be homogenized using a Brinkmann PT 10–35 tissue homogenizer equipped with a saw-tooth PT 20ST probe generator, machine power level set to 4 and homogenized for about 5–10 s.

4 Measurements of microbial biomass should be done on fresh soil samples as quickly as possible (within hours) after sampling, without subjecting the samples to changes in temperature or moisture content. If it is not possible to complete the sample analysis immediately, soil samples should be quickly frozen and kept frozen (−18°C), and then thawed just prior to analysis. However, freezing soil samples may cause changes to the microbial biomass and to the extractability of nonbiomass organic matter (Winter et al. 1994). Ross (1991) has stored soil samples at 4°C with minimal change in microbial biomass.

5 Fumigation–extraction method is relatively precise; three replicate determinations on the same soil sample should be able to detect differences in microbial biomass of 5%–10% at a 0.05% level of probability.

6 Biomass P fumigation–extraction method is extremely sensitive to changes in operating conditions and unless care is taken, reproducible results will not be obtained. It is critical that the extractions be done at the same temperature, shaking speeds, and on the same shaker each time if results are to be compared between soils. It is also important to adopt a standard method of filtration. The one we recommend is to swirl the flask briskly to bring soil particles into suspension, and then to quickly pour the soil extract onto the filter paper. If a ''top up'' of the extract is required, proceed in exactly the same way. Avoid excessive evaporation during the filtration procedure.

7 Microbial biomass C and N assays can be made even more rapidly by substitution of the 24 h $CHCl_3$ fumigation procedure with the addition of liquid $CHCl_3$ to the extracting solution. The liquid $CHCl_3$, 1–2 mL, is added to suspensions of soil in extracting solution just prior to shaking. The use of liquid $CHCl_3$ is preferred for analysis of microbial biomass in substrates containing more than 20% organic matter, e.g., manures and composts. After filtration, $CHCl_3$ is expelled by bubbling CO_2-free air through the filtrate for 1–2 min. While this method greatly simplifies the method, substantially reduces assay time, and is as accurate as the

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fumigation direct-extraction method, less microbial biomass is recovered in the extract ($k_{EC} = 0.18$) (Gregorich et al. 1990). This may be an important factor if microbial biomass levels are low.

8 Soil microbial biomass has an average C:N:P:S ratio of ~100:12:2:1 (Smith and Paul 1990).

We have included values of k_{EC}, k_{EN}, k_{EP}, and k_{ES} for the calculation of microbial biomass C, N, P, and S based on the increase in these elements due to $CHCl_3$ fumigation. While we are reasonably confident in the values of microbial biomass extraction efficiency proposed, it is important to appreciate that there is substantial variation in the literature for different soil organisms and across soil types. Most of the values reported in the literature have been obtained using a limited number of laboratory-grown microbial cultures (mostly bacteria) (Saggar et al. 1981; Brookes et al. 1982; Wu et al. 1994) and with *in situ* calibration techniques (Voroney and Paul 1984; Sparling and West 1988b; Bremer and van Kessel 1990). The method has also been calibrated against alternative methods to estimate microbial biomass (Joergensen 1996; Joergensen and Mueller 1996). However, until more research is done to determine extraction efficiencies for a broad range of soil microorganisms, we recommend that the k_{EC}, k_{EN}, k_{EP}, and k_{ES} values used in the microbial biomass calculations be reported in the methodology.

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Chapter 50
Carbohydrates

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50.1 INTRODUCTION

Carbohydrates represent about 10%–15% of total soil organic carbon (Cheshire 1979) and 5%–10% of organic nitrogen (Greenfield 2001). Most carbohydrates are present in soil as complex polysaccharides composed of monomers of either plant or microbial origin (e.g., cellulose, hemicellulose, peptidoglycan, chitin) (Oades and Wagner 1971; Parsons 1981). Carbohydrates can be used by soil microflora as energy and nutrient sources (Alexander 1977; Cheshire 1979), and have been shown to play a key role in the formation and stabilization of soil aggregates (Cheshire 1979; Tisdall and Oades 1982).

Acid hydrolysis is commonly used to extract polysaccharides from soil (e.g., Ivarson and Sowden 1962; Cheshire and Mundie 1966). This procedure breaks down polysaccharides into monosaccharides, which can be quantified using colorimetric methods that include reactions with anthrone (Brink et al. 1960), alkaline-ferricyanide (Cheshire 1979), phenol–sulfuric acid (Lowe 1993), or Ehrlich's reagent (Stevenson 1982a). Acid hydrolysis of the whole soil yields monosaccharides of different structures, but does not distinguish their source compounds. Chromatographic determination of individual neutral sugars has been used to evaluate the relative contribution of plant and microbes to soil carbohydrates (Oades and Wagner 1971; Puget et al. 1999; Chantigny et al. 2000).

Amino sugars are mostly derived from soil microbes (Parsons 1981; Stevenson 1982b), and their chromatographic quantification has been proposed to evaluate the relative abundance of bacteria and fungi in soils (Zelles 1988) with the knowledge that they are present in both the living and necrotic biomasses (Parsons 1981; Amelung 2001). The most common amino sugars occurring in soils are glucosamine, galactosamine, mannosamine, and muramic acid. Muramic acid is exclusively of bacterial origin, whereas chitin produced by fungi is a dominant source of glucosamine in soil (Parsons 1981; Amelung 2001). Therefore, glucosamine and muramic acid have been used as indicators of the relative contributions of fungi and bacteria to soil organic matter accumulation, and aggregate formation and stabilization (Chantigny et al. 1997; Guggenberger et al. 1999; Six et al. 2001).

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Individual monosaccharides can be quantified by gas chromatography (Oades et al. 1970; Benzing-Purdie 1981; Zhang and Amelung 1996). Although highly sensitive and specific, gas chromatography is time-consuming and requires complex derivatization procedures to transform the hydrolyzed monomers into volatile components. High-performance liquid chromatography (HPLC) can also be used to quantify monosaccharides and is less time-consuming (Angers et al. 1988; Zelles 1988). Recent developments have improved the sensitivity and specificity of HPLC methods (Martens and Frankenberger 1991; Appuhn et al. 2004). In this chapter, acid hydrolysis procedures to extract soil carbohydrates and two chromatographic methods to quantify individual neutral and amino sugars are presented.

50.2 EXTRACTION OF NEUTRAL SUGARS

Sulfuric acid has been widely used to hydrolyze soil polysaccharides (e.g., Ivarson and Sowden 1962; Cheshire and Mundie 1966). Sugars are found in a variety of polymers which differ in their resistance to acid hydrolysis (Oades et al. 1970; Chantigny et al. 2000). Hot water (Haynes and Francis 1993; Puget et al. 1999) and 0.5 *M* H_2SO_4 (Lowe 1993; Puget et al. 1999) have been proposed to extract the most labile fractions of soil polysaccharides. The use of 2.5 *M* H_2SO_4 has been suggested to hydrolyze most of the noncellulosic polysaccharides (Oades et al. 1970), whereas presoaking in 12 *M* H_2SO_4 followed by hot hydrolysis in 0.5 *M* H_2SO_4 has been used to hydrolyze cellulosic materials (Ivarson and Sowden 1962; Lowe 1993; Puget et al. 1999). A proximate polysaccharide fractionation based on acid resistance can thus be achieved using a series of acid hydrolysis of increasing strength (Chantigny et al. 2000).

50.2.1 Materials and Reagents

1. 12 *M* H_2SO_4. This solution is prepared by slowly adding 667 mL of concentrated H_2SO_4 (96% w/w, reagent grade) to 300 mL of deionized water in a 1 L volumetric flask. Let the solution cool to room temperature and bring to 1 L with deionized water.

2. 2.5 *M* H_2SO_4. This solution is prepared by slowly adding 139 mL of concentrated H_2SO_4 to 850 mL of deionized water in a 1 L volumetric flask. Let the solution cool to room temperature and bring to 1 L with deionized water.

3. 0.5 *M* H_2SO_4. This solution is prepared by diluting 28 mL of concentrated H_2SO_4 in 950 mL of deionized water in a 1 L volumetric flask. Let the solution cool to room temperature and then dilute to 1 L with deionized water.

4. Deionized water.

5. Oven, vortex, centrifuge, glass rod.

6. 50 mL polypropylene centrifuge tubes, with screw caps.

7. 250 mL polypropylene centrifuge bottles, with screw caps.

8. Plastic vials of the appropriate volume for sample storage.

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50.2.2 PROCEDURES

Hot-Water Extractable Carbohydrates

1. Weigh 2 g of air-dried, finely ground (<0.15 mm) soil in a polypropylene tube. Air-drying and grinding of the soil before hydrolysis optimize carbohydrate recovery from the soil.
2. Gently add 30 mL of deionized water. Mix on a vortex.
3. Seal tubes with screw caps; incubate for 24 h in an oven set at 85°C.
4. Thoroughly mix the tubes with a vortex and then cool to room temperature.
5. Centrifuge at 16,000 *g* for 10 min.
6. In a second polypropylene tube, transfer 35 parts of the hydrolysate and add one part of concentrated H_2SO_4 (96% w/w, reagent grade).
7. Mix on a vortex and incubate again at 85°C for 24 h. Acidification (step 6) and a second incubation are required for the complete hydrolysis of di-, tri- and oligosaccharides and subsequent determination of individual monomers.
8. Repeat steps 4 and 5.
9. Transfer about 15 mL of the hydrolysate into a plastic vial and store at −20°C until analysis.

Mild-Acid Extractable Carbohydrates

Dilute acid solutions are also used to hydrolyze carbohydrates and are thought to solubilize labile polysaccharides such as microbial materials and hemicellulose. The most often used solutions are 0.5 *M* (e.g., Lowe 1993) and 2.5 *M* H_2SO_4 (e.g., Oades et al. 1970).

1. Proceed as for hot-water extractable carbohydrates, but use 30 mL of either 0.5 *M* or 2.5 *M* H_2SO_4 solution in step 2.
2. Omit steps 6 to 8 as the hydrolysis conditions are already acid.

Strong-Acid Extractable Carbohydrates (Modified from Cheshire and Mundie 1966; Oades et al. 1970)

This extraction procedure includes an initial soaking period, which is aimed at ''softening'' the crystalline forms of polysaccharides, such as cellulose and lignocellulose:

1. Weigh 2 g of air-dried, finely ground (<0.15 mm) soil into a 50 mL centrifuge tube.
2. Add 8 mL of 12 *M* H_2SO_4 and gently mix the soil and acid with a glass rod to ensure that all the soil is moistened with the acid.
3. Loosely cap the tubes and let stand on the bench for 2 h.

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4 Transfer the slurry into a 250 mL polypropylene bottle by washing the tube with 184 mL of deionized water to bring the solution to 0.5 *M* H_2SO_4.

5 Seal bottles and incubate for 24 h in an oven set at 85°C.

6 Thoroughly mix the bottles on the vortex and let cool at room temperature.

7 Centrifuge at 16,000 *g* for 10 min. Transfer about 15 mL in a plastic vial and store at –20°C until analysis.

50.2.3 Comments

1 Acid hydrolysis of neutral sugars has been traditionally performed under reflux (100°C–120°C). However, several investigations have been conducted with sealed tubes. Although some authors have reported lower carbohydrate recovery with sealed tubes relative to reflux (Oades et al. 1970), a detailed direct comparison has not been made. The advantages of using sealed tubes, as compared to reflux, are that sealed tubes are easier to use and more samples can be processed.

2 Performing weak acid (0.5 *M* H_2SO_4) instead of hot-water extraction will yield more neutral sugars (Puget et al. 1999; Chantigny et al. 2000). In general, hot-water extracts have been found to be enriched in microbial sugars (mannose, galactose), originating from exopolysaccharides, which may be involved in soil aggregate stabilization (Feller et al. 1991; Haynes and Francis 1993).

3 Mild- and strong-acid extractable carbohydrates are sometimes termed ''noncellulosic'' and ''cellulosic'' carbohydrates, respectively. However, this terminology is an oversimplification, because once plant residues are incorporated into soil they are rapidly colonized and decomposed by microbes, and therefore both microbial- and plant-derived sugars appear in the extracts (Puget et al. 1999; Chantigny et al. 2000).

4 Sugars are present in soils as a complex mixture of polysaccharides with glycosidic linkages having different degrees of resistance to acid hydrolysis. Prolonged hydrolysis can result in the degradation of some carbohydrates (Martens and Loeffelmann 2002). Any acid hydrolysis procedure is a compromise between maximum release of sugars and minimal decomposition (Greenfield 2001). Where possible, sequential hydrolysis of increasing strength may be the best approach for a thorough characterization and accounting of neutral sugars. However, performing only one of the proposed procedures should prove useful in assessing changes in amounts or composition of sugars over time, or for comparative studies.

50.3 EXTRACTION OF AMINO SUGARS (MODIFIED FROM ZELLES 1988)

50.3.1 Materials and Reagents

1 Glass test tubes (25×100 mm) with Teflon-lined screw caps.

2 Multi-port bubbling system with tubing and needles to insert into the glass tubes.

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3 N_2 gas to supply in the bubbling system.

4 6 *M* HCl. Prepare this solution by diluting 500 mL of concentrated HCl in 475 mL of deionized water in a 1 L volumetric flask. Allow the solution to cool to room temperature and bring to 1 L with deionized water.

5 Oven, vortex, centrifuge.

6 Plastic vials of the appropriate volume for hydrolysate storage.

50.3.2 Procedures

1 Weigh 1 g of air-dried, finely ground (<0.15 mm) soil in a glass tube. Air-drying and grinding of the soil before hydrolysis optimize the amino sugar recovery from the soil.

2 Add 20 mL of 6 *M* HCl. Thoroughly mix on a vortex.

3 Bubble the mixture with N_2 gas for 1 min to remove O_2 from the solution. Bubbling intensity must be carefully adjusted to control foaming and avoid the loss of solution.

4 Seal tubes with screw caps.

5 Incubate the tubes for 6 h in an oven set at 105°C; cool the tubes on ice to room temperature.

6 Carefully decant 10–15 mL of the hydrolysate into a plastic vial. Alternatively, the hydrolysate may be filtered through a glass fiber filter (e.g., Whatman GF/A) if suspended material is present. Store vials at −20°C until analysis.

50.3.3 Comments

Amino sugar extraction has most often been performed using 6 *M* HCl. However, as mentioned for neutral sugars, hydrolysis conditions must be aimed at maximizing amino sugar yields, while minimizing their decomposition. Though this can be performed under reflux (Greenfield 2001), hydrolysis in sealed tubes for 6 h at 105°C has been commonly used as a simple and efficient method for processing large number of samples (Amelung 2001; Appuhn et al. 2004). Bubbling N_2 through the tubes prior to heating is necessary to remove O_2 and avoid possible oxidation of amino sugars during hydrolysis (Zelles 1988).

50.4 Analytical Procedures—Neutral Sugars (Modified from Martens and Frankenberger 1991)

50.4.1 Materials and Reagents—Purification of Extracts

1 Strong-anion solid phase exchange (SAX) resin (3 mL Supleclean LC-SAX columns are available from Supelco, Inc., Bellefonte, PA).

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2 Strong-cation solid phase exchange (SCX) resin (3 mL Supelclean LC-SCX columns are available from Supelco, Inc., Bellefonte, PA).

3 Solution of 0.5 *M* NaOH, prepared by dissolving 2 g of NaOH in 90 mL of deionized water in a 100 mL volumetric flask. Then, dilute to volume.

4 50 mL glass beakers.

5 Filter papers (Whatman #42).

6 Plastic vials of appropriate volume for storage.

7 1.5 mL Eppendorf centrifuge tubes.

50.4.2 Purification Procedures

1 Transfer 5 mL of any hydrolysate obtained in Section 50.2 in a 50 mL beaker.

2 Slowly add 0.5 *M* NaOH solution to neutralize to pH 6.5–7.0. Record the volume of NaOH solution added to correct for dilution of the hydrolysate and sugar content calculations.

3 Filter the solution (Whatman #934-AH) and collect filtrate in a plastic vial. The neutralized filtrate can be stored at −20°C until analysis.

4 Pass a sufficient volume (generally about 4 mL are sufficient for analytical purposes) of the neutralized filtrate through the SAX resin and collect the cation-purified solution in a beaker.

5 Pass the cation-purified solution through the SCX resin and collect the purified solution in a 1.5 mL Eppendorf tube; store the tubes at −20°C until analysis.

50.4.3 Comments

1 In all cases, soil hydrolysates must be purified prior to chromatographic analysis of neutral sugars to remove any interfering ionic compounds. This purification is the most time-consuming part of the process to measure soil neutral sugars. Using SCX and SAX resins to purify hydrolysates requires conditioning and cleaning of the resins between samples. The conditioning and cleaning procedures are supplied by the resin manufacturer and must be strictly followed to ensure that the columns function correctly. We found that purifying hydrolysate through SAX before SCX extended the life of the resins. When appropriately conditioned and cleaned, the LC-SAX and LC-SCX columns supplied by Supelco can be used to purify three to five samples before being discarded; purification efficiency of these columns is optimized if the hydrolysate flow rate is less than 5 mL per min.

2 Including blank samples during the extraction and purification process is advisable especially when cellulose-based filter papers are used. These filter papers may release some arabinose, xylose, and glucose, which can then be corrected for with blank samples.

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50.4.4 Materials and Reagents—Analytical Procedures

1. High-performance anion exchange chromatograph with pulsed amperometry detector (HPAEC-PAD) (Model DX-500, available from Dionex, Sunnyvale, CA). This system must be equipped with a gradient pump, an electrochemical detector, and an autosampler.

2. Detector cell with disposable gold electrode.

3. CarboPac-PA10 precolumn (4×50 mm) in series with an analytical column (4 × 250 mm).

4. 10 to 50 μL injection loops. The volume of the loop to be used is reduced as sugar content increases in the samples.

5. Two eluent organizers and two 2 L eluent containers to store and deliver eluents to the system.

6. 0.5 mL PolyVials with filter caps (available from Dionex) to prepare samples for injection through the autosampler.

7. NaOH solution (50% w/w, available from Fisher Scientific).

8. Nanopure water (specific resistance of 18 MΩ). Prior to use, nanopure water is degassed by filtering (0.45 μm) under vacuum.

50.4.5 Chromatographic Conditions

1. Eluent A: 21 m*M* NaOH: Pour degassed nanopure water in a 2 L volumetric flask. Add 2.1 mL of 50% NaOH solution and dilute to volume. Immediately transfer into a 2 L eluent container and seal the container. Keep the headspace under He atmosphere to avoid carbonate formation.

2. Eluent B: 500 m*M* NaOH: Pour degassed, nanopure water in a 2 L volumetric flask. Add 52.8 mL of 50% NaOH solution and dilute to volume. Immediately transfer into a 2 L eluent container and seal the container. Keep the headspace under He atmosphere to avoid carbonate formation.

3. Set the electrochemical detector mode to ''pulsed amperometry.''

4. Set working pulse potentials (E) and durations (t) to: $E_1 = 0.1$ V, $t_1 = 300$ ms; $E_2 = 0.6$ V, $t_2 = 120$ ms; $E_3 = -0.6$ V, $t_3 = 60$ ms.

5. Transfer about 0.7 mL of the purified sample obtained in step 5 of Section 50.4.2 to a 0.5 mL PolyVial (a PolyVial has a total volume of 1 mL). After inserting the filter cap, only 0.5 mL of sample remains, and the excess sample volume (about 0.2 mL) passes through the filter cap and is wiped off before entering the vial in the autosampler.

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TABLE 50.1 System Schedule for Determination of Neutral Sugars in Soil Extracts by High-Performance Anion Exchange Chromatography with Pulsed Amperometry Detection (HPAEC-PAD)

Run time (min)	Step description	Total flow rate (mL min^{-1})	% Eluent A	% Eluent B
0.0	Conditioning	1.0	0	100
13.1	Equilibration	1.0	100	0
22.0	AutoSampler ON	0.8	100	0
23.1	Sample injection	0.8	100	0
43.1	End of run	0.8	100	0

Eluent A: 21 m*M* NaOH solution; Eluent B: 500 m*M* NaOH solution. Solutions must be kept under He atmosphere to prevent carbonate formation.

6 Chromatographic schedule (isocratic analytical condition) is set as shown in Table 50.1.

Under these chromatographic conditions, seven neutral sugars can usually be identified (Figure 50.1).

50.4.6 Comments

1 High-performance liquid chromatography has been proposed to quantify neutral sugars in soil hydrolysates (Angers et al. 1988). However, HPAEC with PAD (HPAEC-PAD) has proven more sensitive and specific for several neutral sugars (Martens and Frankenberger 1991).

2 CarboPac-PA10 column is highly sensitive to the presence of carbonates and other soil contaminants, which decrease sugar retention time, especially for sugars

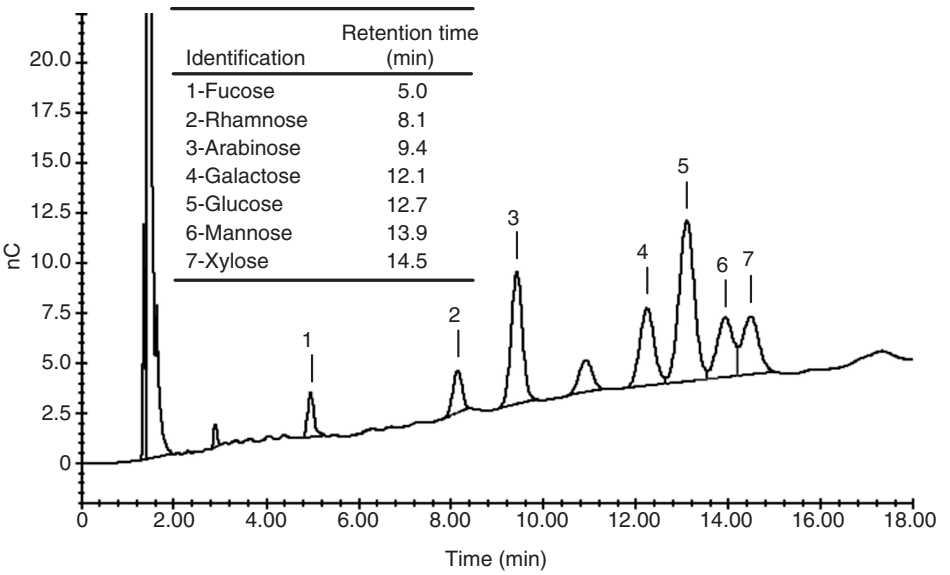

FIGURE 50.1. Typical chromatogram for neutral sugars in a soil extract measured with HPAEC-PAD.

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eluting later on the chromatogram. Even though hydrolysates are purified prior to analysis, the sugar retention times will gradually decrease as the number of consecutive injections increases. To avoid identification problems, we recommend that a standard sample be injected every 5 to 10 injections. Alternatively, increasing the duration of the conditioning step (Table 50.1) will also help clean the column and maintain retention times. Renewal of eluents once a week is also advisable to avoid carbonate accumulation in the eluent containers.

50.5 ANALYTICAL PROCEDURES—AMINO SUGARS (MODIFIED FROM ZELLES 1988)

50.5.1 Materials and Reagents

1. High-performance liquid chromatograph equipped with a gradient pump, column-heating device, fluorescence detector, and a manual injection port (in our laboratory we use a HPLC manufactured by Waters Limited, Milford, MA operated under the Millenium system).

2. Analytical column: NOVA-PACK C18, 4 μ, 8 × 100 mm (Waters column #8 NVC18, 4 μ).

3. Guard column: NOVA-PACK C18 4 μ.

4. Rotary vacuum evaporator with heated water bath, and 25 mL boiling flasks.

5. Microcentrifuge to fit 1.5 mL Eppendorf centrifuge tubes.

6. 0.5 *M* potassium tetraborate solution: Dissolve 30.55 g of potassium tetraborate in 200 mL of deionized water. Adjust at pH 10.0, if required, with 1 *M* NaOH. Store at room temperature in a sealed glass bottle.

7. *O*-Phthaldialdehyde (OPA) solution: Dissolve 250 mg of 99% pure OPA (available from Sigma) in 50 mL of 0.5 *M* potassium tetraborate. Store the solution at 4°C.

8. 2-Mercaptoethanol.

9. Eluent A: Sodium citrate/acetate–methanol–THF. This eluent is prepared by dissolving 25.59 g of sodium citrate in 870 mL of nanopure water (specific resistance of 18 MΩ). In another beaker, dissolve 11.84 g of sodium acetate in 870 mL of nanopure water. Then mix both solutions together and adjust pH to 5.3 with glacial acetic acid. Finally, add 164 mL of methanol and 96 mL of tetrahydrofuran (THF). Mix the solution well and vacuum filter (0.45 μm) to degas the eluent. Store in amber glass bottle.

10. Eluent B: 65% methanol. Measure separately 650 mL of methanol (HPLC grade) and 350 mL of nanopure water. Vacuum filter (0.45 μm) each solution separately, then mix and store in a glass bottle.

11. Column cleaning solution: 100% methanol. Vacuum filter (0.45 μm) HPLC grade methanol and store in a glass bottle.

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50.5.2 Sample Preparation and Derivatization

1 In a 25 mL boiling flask, transfer a 1 mL aliquot of the hydrolysate obtained in step 6 of Section 50.3.2, and evaporate to dryness with the rotary vacuum evaporator.

2 Add 980 μL of OPA solution and 20 μL of 2-mercaptoethanol to the flask and mix on a vortex. The derivatization period starts when mercaptoethanol is added.

3 Transfer as much material as possible in a 1.5 mL Eppendorf tube, and centrifuge at ca. 20,000 *g* for 2–3 min.

4 Collect 25 μL of supernatant with the appropriate injection syringe. Inject the sample into the HPLC when the derivatization period reaches 5 min (±15 s) and start the HPLC run. Discard the unused derivatized sample.

50.5.3 Chromatographic Conditions

1 Analytical column temperature: 40°C.

2 Detection: Fluorescence-OPA with emission wavelength set at 425 nm and excitation wavelength set at 338 nm.

3 Detector lamp type: Xenon.

4 Injection volume: 25 μL.

5 Chromatographic schedule (gradient type) (see Table 50.2).

Under these chromatographic conditions, four amino sugars can usually be identified (Figure 50.2).

Important note: The analytical column must be cleaned once a day using the following schedule:

1 Total eluting flow rate is set to 1.5 mL min^{-1}. Gradually (approximately over 2 min) increase the proportion of Eluent B from 15% to 100%. Let stand for at least 5 min.

TABLE 50.2 System Schedule for Determination of Amino Sugars in Soil Extracts by High-Performance Liquid Chromatography (HPLC)

Run time (min)	Step description	Total flow rate (mL min^{-1})	% Eluent A[a]	% Eluent B
0.0	Injection[b]	1.5	85	15
2.5	Gradient phase	1.5	85	15
17.5	Gradient phase	1.5	70	30
20.0	End of gradient	1.5	85	15
25.0	Conditioning	1.5	85	15

[a] Eluent A: sodium citrate/acetate–methanol–THF solution; Eluent B: 65% methanol in water.
[b] At 5 min ±15 s after start of the derivatization period (starts when adding 2-mercaptoethanol to the sample).

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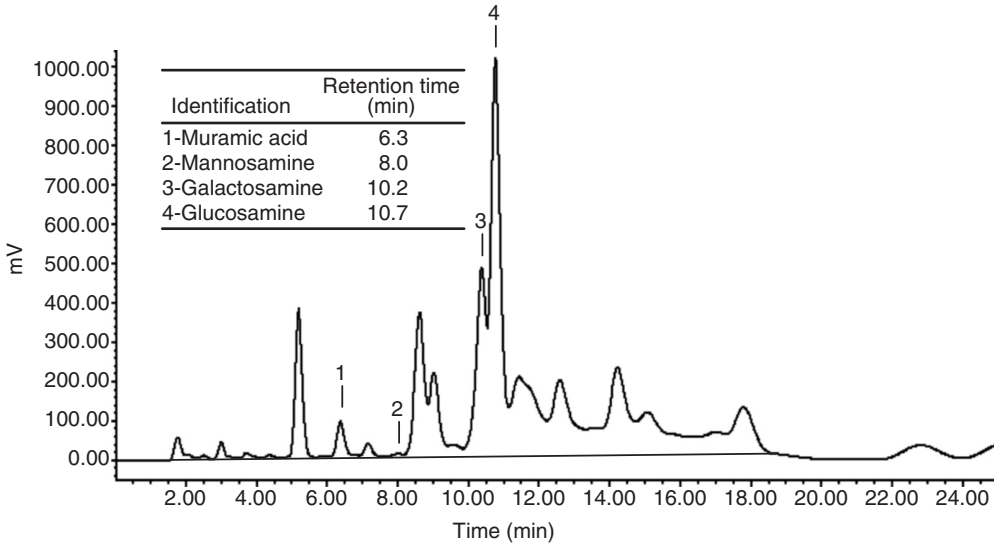

FIGURE 50.2. Typical chromatogram for amino sugars in a soil extract measured with HPLC.

2 Switch from Eluent B to the cleaning solution (100% methanol). Let stand for at least 10 min.

3 Switch back to Eluent B. Let the column equilibrate for 10 min. Gradually (approximately 2 min) increase the proportion of Eluent A back to analytical conditions. Let stand for 15 min before injecting a new sample.

50.5.4 Comments

1 Colorimetric determination of total amino sugars in soil is possible using Ehrlich's reagent (Stevenson 1982a). However, chromatographic analysis is required to determine the abundance of individual amino sugars. The analytical method proposed here was modified from the original HPLC method of Zelles (1988) and is similar to that of Appuhn et al. (2004). However, the chromatographic run of the method we propose is much shorter, does not require a thermostatic regulated autosampler, and the derivatization and injection of the sample into the chromatograph can be done manually.

2 Fluorescent complex formed between amino groups and OPA in the presence of mercaptoethanol is transient (Amelung 2001) and therefore must be the same for all samples. Appuhn et al. (2004) proposed to use an automated derivatization-injection system. However, manual injection is also acceptable provided that the time elapsed between adding the 2-mercaptoethanol to the sample and injecting the sample into chromatograph is kept constant. In our laboratory, a period of 5 min ($\pm$15 s) has been found to be suitable for obtaining a maximum and consistent fluorescence response from amino sugars.

3 Fluorescence emission varies among amino sugars with the strongest values (lowest detection limits) for muramic acid followed in decreasing order by glucosamine, galactosamine, and mannosamine (Appuhn et al. 2004).

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Chapter 51
Organic Forms of Nitrogen

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51.1 INTRODUCTION

Determination of the chemical forms of soil organic nitrogen (N) has most commonly involved a two-step procedure. First the soil is hydrolyzed by hot mineral acid (usually hydrochloric acid [HCl]), and second the hydrolysate is either distilled to isolate operationally defined N fractions or it is chromatographically separated for identification of common amino acids (Bremner 1965; Stevenson 1994). Normally this approach identifies at most half of the total soil N. This poor recovery may be explained by several factors: HCl hydrolysis does not solubilize a considerable proportion (~20%–35%) of total soil N, a comparable proportion is hydrolyzed from various N forms into NH_4^+, which obscures the original forms, and ~10%–20% of total soil N is hydrolyzable but not identifiable as any of the commonly measured amino acids (Stevenson 1994, 1996).

The acid-insoluble N and the hydrolyzable unknown N are often assumed to consist of organic N compounds more recalcitrant than amino acids, such as heterocyclic N, leading to speculation that the general recalcitrance of soil organic N is due to an abundance of aromatic structures (Flaig et al. 1975; Schulten and Schnitzer 1998). Clear evidence supporting this speculation, though, has not yet been found, and the chemical forms of acid-insoluble N and hydrolyzable unknown N remain uncertain. Much of the acid-insoluble N may be secondary amide N—polypeptides, amino acids, amino sugars, and their residues—that is resistant to acid hydrolysis (Knicker and Hatcher 1997; Nguyen and Harvey 1998).

Newly developed spectroscopic techniques are enabling greater insights into the various forms of soil organic N. Involving advanced instrumentation, these analyses are demonstrating that the bulk of soil organic N is amide, i.e., aliphatic, and that aromatic N is indeed present, but it constitutes at most a modest proportion of soil N. Most evidence has been gained through ^{15}N nuclear magnetic resonance (NMR) spectroscopy using cross-polarization magic-angle spinning (CP/MAS), which in nearly all studies found negligible signals for all N forms other than amide N (Preston 1996; Knicker et al. 1997). Questions remain as to whether the large amide N signal gained by CP/MAS ^{15}N NMR obscures smaller signals for heterocyclic N and whether many forms of heterocyclic N compounds exist in the soil but at

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levels too low for clear detection by CP/MAS ^{15}N NMR (Schulten and Schnitzer 1998; Thorn and Mikita 2000).

Other new techniques performed on limited numbers of samples detected modest levels of heterocyclic N compounds, ranging from <10% to ~30% of total N in soil, humic fractions, or decomposing plant litter. These techniques include pyrolysis (Schulten et al. 1995; Bracewell et al. 1980; Olk et al. 2002), nitrogen x-ray absorption near-edge structure (Vairavamurthy and Wang 2002; Jokic et al. 2004), x-ray photoelectron spectroscopy (Abe and Watanabe 2004; Abe et al. 2005), and ^{15}N NMR (Skene et al. 1997; Knicker and Skjemstad 2000; Mahieu et al. 2000). The identified heterocycles included pyridine, pyrrole, and indole.

Despite considerable effort, only very recently has evidence been obtained for the presence in soil organic matter of anilide-like N compounds, which readily form under laboratory conditions through covalent binding of organic N with phenolic compounds. Saturation-pulse-induced dipolar exchange with recoupling (SPIDER) is an advanced NMR technique to selectively observe carbon (C) forms that are bonded to N (Schmidt-Rohr and Mao 2002). Using SPIDER on one soil, Schmidt-Rohr et al. (2004) identified ~20% of soil organic C as being aromatic and bound to N, including <8% as anilides. For a phenol-enriched humic fraction that was extracted from this soil, 25% of the humic N was anilide N and 18% was heterocyclic N. Analysis by ^{15}N NMR of N forms in this humic sample found predominantly secondary amide (Mahieu et al. 2000). Hence the anilide N observed by SPIDER was detected by ^{15}N NMR as amide N, placing in question the capacity of ^{15}N NMR to quantitatively detect anilide N and perhaps also heterocyclic N. The SPIDER analysis is not yet widely available and requires NMR spectrometers with triple resonance.

Amino sugars are thought to account for up to 10+% of total soil N, based on the traditional acid hydrolysis and steam distillation techniques (Parsons 1981; Stevenson 1994) and newer procedures that use gas chromatography, high-performance liquid chromatography (HPLC), or amino acid analyzers with pre- or postcolumn derivatization (Amelung 2001). A recommended procedure for determining amino sugar concentrations is provided in Chapter 50.

Martens and Loeffelmann (2003) and Martens et al. (2006) introduced an analysis for amino acids that simultaneously measures the amino sugars glucosamine and galactosamine and does not require chemical derivatization. With this procedure the soil is hydrolyzed with methanesulfonic acid (MSA) instead of HCl, which avoids the HCl-induced oxidation of S-containing amino acids and degradation of serine and threonine. Also, MSA is nonvolatile and thermally stable at elevated temperatures. Nitrogen forms were detected by anion chromatography and pulsed amperometry, which measures the electrical current produced upon oxidation of each amino compound. This approach identified 51% of total N in several parts of United States Midwestern soils (Martens and Loeffelmann, 2003; Martens et al. 2006), mostly as amino acids with smaller amounts of amino sugars. Another 35% of total soil N was recovered in the hydrolysate as NH_4^+. By comparison, HCl hydrolysis of 12 of these soils followed by anion chromatographic detection identified 47% of total soil N (Martens and Loeffelmann 2003). To date few published studies have used anion chromatography and pulsed amperometry to measure soil N forms.

As discussed above, none of the currently available techniques for quantifying organic N forms in soils achieves a very high recovery or a complete characterization of the organic N forms. Nevertheless, relatively standard techniques for characterizing organic N have been defined and are in regular use. In this chapter, we focus on describing chromatographic

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techniques and steam distillation for analysis of organic N forms in hot HCl hydrolysates of soils. A brief description is also given for amino acid and amino sugar analysis by anion chromatography and pulsed amperometry following MSA hydrolysis of soil.

51.2 ANALYSIS FOR NITROGEN FORMS BY HCl HYDROLYSIS FOLLOWED BY STEAM DISTILLATION OR CHROMATOGRAPHY

51.2.1 Preparation of Soil Hydrolysates (Bremner 1965)

Hydrolysis is conducted under reflux with HCl for about 12 h, using 3 mL of 6 *M* HCl/g soil, and the soil hydrolysate is neutralized without prior removal of excess acid.

Materials and Reagents

1. Micro-Kjeldahl digestion unit.
2. Liebig condensers with 24/40 ground-glass joint.
3. Round-bottom flasks fitted with a standard-taper (24/40) ground-glass joint as for the Liebig condenser.
4. Electric heating mantle.
5. Hydrochloric acid, approximately 6 *M*: add 513 mL of concentrated HCl (specific gravity 1.19) to about 500 mL of water, cool, and dilute to 1 L in a volumetric flask.
6. *N*-Octyl alcohol.
7. Sodium hydroxide, approximately 10 *M*: place 3.2 kg of reagent-grade NaOH in a heavy-walled 10 L Pyrex bottle marked to indicate a volume of 8 L. Add 4 L of CO_2-free water and swirl the bottle until the alkali is dissolved. Cool the solution while the neck of the bottle is closed with a rubber stopper, and then dilute it to 8 L by the addition of CO_2-free water. Swirl the bottle vigorously to mix the contents and fit the neck with some arrangement that permits the alkali to be stored and dispensed with protection from atmospheric CO_2.
8. Sodium hydroxide, approximately 5 *M*: dilute 500 mL of 10 *M* NaOH to 1 L and store in a stoppered bottle.
9. Sodium hydroxide, approximately 0.5 *M*: dilute 50 mL of 10 *M* NaOH to 1 L and store in a stoppered bottle.

Procedures

1. Place a sample of finely ground (<100 mesh) soil containing about 10 mg of N in a round-bottom flask fitted with a standard-taper (24/40) ground-glass joint.
2. Add two drops of octyl alcohol and 20 mL of 6 *M* HCl, then swirl the flask to thoroughly mix the acid with the soil.

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3 Place the flask in an electric heating mantle and connect it to a Liebig condenser fitted with a 24/40 ground-glass joint.

4 Heat the soil–acid mixture so that it gently boils under reflux for 12 h.

5 Wash the condenser with a small quantity of distilled water and allow the flask to cool, and then remove the flask from the condenser.

6 Filter the hydrolysis mixture through a Buchner funnel fitted with Whatman No. 50 filter paper, using a suction filtration apparatus that allows collection of the filtrate in a 200 mL beaker.

7 Wash the residue with distilled water until the filtrate reaches the 60 mL mark on the beaker.

8 Place the bottom half of the beaker in crushed ice.

9 Neutralize to pH 6.5 $\pm$ 0.1 by slow addition of NaOH with constant stirring to ensure that the hydrolysate does not become alkaline at any stage of the neutralization process. Use 5 *M* NaOH to bring the pH to about 5, and then complete the neutralization using 0.5 *M* NaOH. The hydrolysate can also be cooled in a freezer and cold NaOH used for neutralization.

10 Transfer the neutralized hydrolysate into a 100 mL volumetric flask.

11 Adjust the volume to the mark with the washings obtained by rinsing the beaker, electrodes, and stirring device several times with small quantities of distilled water.

12 Stopper the flask and invert several times to mix the contents.

51.2.2 Measurement of N Compounds in the Acid Hydrolysate

Nitrogen Fractionation Based on Steam Distillation

Nitrogen fractions in the acid hydrolysate have often been distinguished through steam distillation, as described by Stevenson (1996). Nitrogen fractions include total hydrolyzable N, acid-insoluble N, amino acid-N, ammonia-N, amino sugar-N, and hydrolyzable unknown N (Table 51.1). Further use of this fractionation scheme should be questioned as its fractions appear largely irrelevant to N cycling under field conditions (Stevenson 1982), it may have methodological errors (Mulvaney and Khan 2001), and it does not distinguish individual amino compounds.

Analysis for Amino Acids and Amino Sugars by Chromatographic Separation

Several chromatographic methods are available for determining the concentrations of individual amino acids and amino sugars in HCl hydrolysates. To measure the amino acids in hydrolysates of pure proteins, biochemists often combine reverse-phase HPLC with precolumn derivatization and fluorescence detection (Cooper et al. 2001). This approach enables

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TABLE 51.1 Steam Distillation Methods for Determining the Various Forms of N in a Soil Hydrolysate

Form of N	Method[a]
Total hydrolyzable N	Steam distillation with NaOH after Kjeldahl digestion with H_2SO_4 and a K_2SO_4-catalyst mixture
Amino acid N	Steam distillation with phosphate–borate buffer after treatment with NaOH at 100°C to remove amino sugars plus NH_4^+ and with ninhydrin (pH 2.5, 100°C) to convert α-amino N to NH_4^+
Amino sugar N	Steam distillation with phosphate–borate buffer at pH 11.2; correction for NH_3-N
Ammonia N	Steam distillation with MgO
Acid-insoluble N	Obtained by difference (total N – hydrolyzable N)
Hydrolyzable unknown N	Obtained from the difference between total hydrolyzable N and the N accounted for as (NH_3 + amino acid + amino sugar)-N

Source: From Stevenson, F.J. in D.L. Sparks et al. (Eds.), *Methods of Soil Analysis, Part 3—Chemical Methods.* Soil Science Society of America, Madison, Wisconsin, 1996, 1185–1200. With permission.

[a] In each method, the NH_3 liberated by steam distillation is collected in a H_3BO_3-indicator solution and determined by titration with standard (0.0025 *M*) H_2SO_4.

sensitive detection of amino acids, but it is not directly applicable to soil due to interference by soil components. Warman and Bishop (1985) adapted reverse-phase HPLC to soil analysis by first dissolving silicate soil minerals with HF and then removing soil metal cations by adsorption onto cation-exchange resin. Jones et al. (2005) used reverse-phase HPLC to measure amino acids in the soil solution. Amelung and Zhang (2001) measured concentrations of each soil amino acid after separation into its two enantiomers, whose molecular structures are mirror-image and nonsuperimposable. Acid hydrolysates were purified of interfering organic compounds by adsorbing the amino acids onto a cation-exchange resin, followed by an oxalic acid wash to remove soil cations. Amino acid enantiomers were then eluted from the resin with NH_4OH, derivatized to form *N*-pentafluoropropionyl-amino acid *iso*-propyl esters, and analyzed by gas chromatography. Amelung and Zhang (2001) reviewed other gas chromatography analyses for soil amino acids. Amelung (2001) described analyses for amino sugars that involved either reverse-phase HPLC or gas chromatography.

By far the most common approach for routine analysis of soil amino acids has been ion-exchange chromatography with post-column derivatization by ninhydrin (triketohydrindane hydrate) (Moore and Stein 1948; Moore et al. 1958). The acid hydrolysate is injected into a liquid chromatograph equipped with a strong cation-exchange column, onto which the amino acids will adsorb, given their positive charge in the acid solution. After other soil components have passed through the column, amino acids are then sequentially eluted from the column by using solution gradients that vary the mobile-phase pH, salt concentration, and temperature. Typically the pH is controlled by varying the level of Na citrate, and salt concentration is controlled by varying the level of NaCl. The eluted amino acids are derivatized with ninhydrin, which binds highly selectively with the α-NH_2 groups of amino acids, peptides, primary amines, and NH_3. The resulting complexes are quantified by measuring their light absorption at both 440 (proline and hydroxyproline) and 570 nm (all other compounds). This method requires a longer run time and is much less sensitive

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than fluorescence detection to amino compounds, but it is not vulnerable to interference by other soil components, some of which can also fluoresce when derivatized.

Ion-exchange amino acid analyzers are available from multiple sources, and specific steps in the chromatographic analysis can differ slightly by manufacturer. Manufacturer's specifications should be followed when developing analytical steps, including the selection of reagents and their gradients. Stevenson (1965) described one procedure for ion-exchange chromatography, but significant advances in the capabilities of chromatography columns (Zumwalt and Gehrke 1988) have made this procedure obsolete.

51.2.3 Comments

1 Acid hydrolysis and steam distillation can be performed with relatively affordable equipment. Chromatographic separation requires more advanced instrumentation, which is often available at major research institutes in specialized units such as protein structure laboratories.

2 As previously described, any procedure that involves HCl hydrolysis of the soil will result in a moderate recovery (~50%) of soil N as identifiable amino acids, because of incomplete solubilization of N compounds, cleavage of some amine groups to form free ammonium, and extensive destruction of specific amino acids.

51.3 ANALYSIS FOR INDIVIDUAL AMINO ACIDS AND AMINO SUGARS BY METHANESULFONIC ACID HYDROLYSIS AND ANION CHROMATOGRAPHY–PULSED AMPEROMETRY

As described above, Martens and Loeffelmann (2003) identified amino acids and amino sugars simultaneously by (i) hydrolyzing soil with MSA instead of HCl and (ii) measuring amino compounds by anion chromatography and pulsed amperometry. Use of MSA allowed preservation of some labile amino acids.

The soil is extracted with MSA during autoclaving, and subsequently the supernatant is neutralized to an alkaline pH, making the amino compounds negatively charged. The supernatant is passed through an anion-exchange column that has quaternary NH_4 groups, enabling highly specific adsorption of amino compounds. The amino compounds are eluted with a tertiary gradient of water, NaOH, and Na acetate. This method requires no derivatization, due to the highly specific binding of amino compounds by the column.

This approach has been used for analysis of model amino compounds (Clarke et al. 1999), proteins (Jandik et al. 2001), and soil amino compounds (Martens and Loeffelmann 2003; Martens et al. 2006). Results from this limited number of studies have shown that the optimal autoclaving duration for maximum extraction of amino compounds must be determined for each soil. The optimal duration can differ by soil type (including clay mineral type and clay quantity) and land use practices such as tillage and animal manure amendment. Martens and Loeffelmann (2003) and Martens et al. (2006) used short autoclaving times (30 to 90 min), but at a temperature of 136°C. Unfortunately, many autoclaves cannot achieve temperatures >121°C. Atmospheric oxygen is not excluded during the extraction, creating the potential for partial degradation of amino sugars (Amelung 2001).

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Chapter 52
Soil Humus Fractions

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52.1 INTRODUCTION

Soil organic matter may be differentiated into humic and nonhumic components. Humic components or humus are the highly transformed, dark brown to black materials that are closely associated with the mineral fraction. Nonhumic material includes both particulate matter (e.g., dead roots) and simple soluble components that are part of the soil solution. Although the definition is straightforward, the consistent separation of humic and nonhumic components is problematic. It is a challenge to develop experimental methods that consistently and definitively separate humic and nonhumic fractions.

MacCarthy (2001) postulated that humic substances are unique in nature and have qualities unlike any other natural substances. The first principle postulates that humic substances represent a supermixture of an extremely wide array of chemical structures. In all likelihood the probability of finding two identical structures is exceedingly remote. By understanding humic substances in this way, it is evident that classical approaches based on isolation, purification, and finally identification of discrete chemical compounds and structures do not apply. This is not to say that extractions should not be used, but that they should be used within the context of this new understanding of the limitations of the classical methods.

Despite concerns about the efficiency of extractions (generally less than one-half of the humus is extracted) and the creation of artifacts during the extraction, extraction and fractionation remain important steps in many studies. Extraction separates the humic materials from the complicating presence of the mineral fraction, removes other inorganic interferences, increases concentration, and renders the organic matter soluble (Swift 1996).

The extraction of organic matter with alkali, the subsequent separation of the extract from the remaining soil, and the acidification of the extract results in three operationally defined fractions: humic acids (HA), fulvic acids (FA), and humin. The FA is the generally yellowish-colored fraction that remains in solution after acidification of the alkaline extract, the HA is the dark brown to black precipitate resulting from the acidification of the extract, and the humin is the material not extracted. About 30% to 60% of the soil humus is removed with alkali extractants.

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Humin, the fraction insoluble in aqueous extracts at any pH or the nonextractable humus, is understandably the least studied and, therefore, the least understood part of the organic matter (Rice 2001) despite its being more than 50% of the organic carbon (C) in most soils. In essence, humin is a small amount of organic matter associated with a considerable mass of soil. Humin, compared to HA, is more aliphatic and has significant aromatic and carbohydrate components. About one-third to one-half of the C remaining with the soil residue following alkali extraction can be isolated by simply adding water, dispersing the dilute suspension with ultrasound, and centrifuging to settle out all but the very fine clay (Anderson et al. 1974). Acidification of the centrifugate results in a dark, humic material, thought to be clay-associated. These humic materials (normally part of the humin) are less aromatic, more susceptible to acid hydrolysis, richer in N, and of higher molecular weight than the humic materials extracted in the first step with strong alkali. After isolation of this second HA fraction, the humin remaining with the soil residue is mainly particulate bits of plant root, fungal remains, and charcoal. A method for isolating humin fractions by partitioning between an aqueous phase of varying pH and methylisobutylketone is recommended to those interested in the humin fraction (Rice 2001).

There are a great number of extraction methods involving many different extractants. The extraction method outlined below uses an alkali extractant and is suitable for research involving comparisons of related groups of soils. The extraction and fractionation described are recommended for use, but with a precautionary principle that recognizes their limitations.

52.2 SAMPLING AND SAMPLE PREPARATION

A first concern in sampling is the separation of humic and nonhumic materials. Recognizable plant residues should be removed from the soil surface before coring. Using a core sampler of known volume is recommended (see Chapter 3), in order to calculate bulk density for use in later data analysis. Because of soil variability, and the laborious nature of extractions, taking several cores and then mixing thoroughly to obtain a composite sample may be a good strategy. Sampling the depth of cultivation (Ap horizon) is recommended, although subhorizons (e.g., 0 to 5 cm and 5 to about 15 cm) will help in evaluating differences due to agronomic treatments. Sampling pedologically defined horizons is recommended although different depths of sampling introduce an additional challenge for statistical analyses (see Chapter 1).

Air-drying samples in a dust-free space is recommended. Air-dry samples should be ground lightly using a rolling pin or similar device, and passed through a 2 mm sieve. During this step, in contrast to methods estimating total organic C contents (see Chapter 3), visible roots and other plant residues should be removed. A representative subsample should be finely ground in preparation for analyses such as organic C or nitrogen (N) determination, thereby reducing variability. Extractions may be done on the larger, <2 mm sample.

52.3 ALKALI EXTRACTION

An alkali extraction is used to remove a portion of the soil organic matter. Alkali extractants which are commonly used include 0.1 *M* NaOH, 0.5 *M* NaOH, and 0.1 *M* NaOH–0.1 *M* $Na_4P_2O_7$. The method below describes the use of 0.5 *M* NaOH.

52.3.1 Reagents

1 0.5 *M* HCl: Prepare the reagent by carefully adding 40 mL of concentrated HCl to deionized water and bring the total volume to 1000 mL, working in a fume hood.

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This reagent is used as a pretreatment to remove floating plant debris and inorganic forms of C, N, phosphorus (P), and sulfur (S) before extraction with NaOH.

2 0.5 *M* NaOH: dissolve 20.0 g of NaOH in 1000 mL of deionized water. The pH of the solution should be around 13.5. The NaOH solution must be prepared fresh daily and kept tightly covered, as it will absorb carbon dioxide from the atmosphere.

52.3.2 Procedure

1 Place 15 g of air-dried soil (ground to <2 mm) into a 250 mL plastic centrifuge bottle that withstands high-speed refrigerated centrifugation.

2 Add 150 mL of 0.5 *M* HCl. Set aside for 1 h, stirring occasionally. Centrifuge for 15 min at 9000 *g* and pour off the supernatant.

3 To wash the soil free of any remaining HCl, add 150 mL of deionized water to the centrifuge bottle, mix, then centrifuge at 9000 *g* for 15 min, and discard the supernatant.

4 Add 150 mL of fresh 0.5 *M* NaOH to the centrifuge bottle. Flush the headspace of the bottle with oxygen-free N_2 gas, and then quickly tighten the cap.

5 Place the bottle on an end-over-end shaker (60 turns per min) for 18 h.

6 Following the shaking, centrifuge at 9000 *g* for 15 min to separate the NaOH extract from the soil residue. Carefully decant the supernatant into a clean centrifuge bottle and retain for separation into HA and FA fractions. The residue of the extraction (humin) may be discarded or retained for analysis (see Section 52.4).

52.3.3 Comments

1 Pretreatment step removes a small amount of organic matter and the dissolved organic carbon (DOC). The International Humic Substances Society (IHSS) method (Swift 1996) includes the DOC with the FA. The pretreatment should be performed if organic nutrients are to be analyzed with minimal interference.

2 Soils containing calcium carbonate will react violently with the 0.5 *M* HCl.

3 Retention of N_2 headspace during extraction is important in reducing oxidation and CO_2 absorption. For this reason, centrifuge bottles should be selected with a good seal between cap and bottle. A wrist-action shaker may be substituted if an end-over-end shaker is not available.

52.4 SEPARATION INTO HUMIC FRACTIONS

The NaOH extract is fractionated into conventional HA and FA fractions by acidification. The residue of the alkali extraction, sometimes referred to as humin, is largely composed of organic material tightly bound to the mineral fraction. The humin may be discarded, retained

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for analysis, or sonified and fractionated into clay-associated HA (HA-B) and FA (FA-B) fractions (Anderson et al. 1974; Bettany et al. 1979).

52.4.1 Reagents

1 6 *M* HCl: Prepare the reagent by carefully adding 50 mL of concentrated HCl to 50 mL of deionized water, working in a fume hood.

2 0.1 *M* NaOH: dissolve 4.0 g of NaOH in 1000 mL of deionized water. This solution is used to redissolve the HA after separation by acidification.

52.4.2 Procedure

1 Using a burette, add 6 *M* HCl to the NaOH extract in the centrifuge bottle until a pH of 1.5 is attained, checking with a pH meter while stirring. The acidification causes precipitation of a portion of the organic matter, that is dark brown to black in color and is termed conventional HA (HA-A), while that which remains in solution after acidification (yellowish in color) is termed conventional FA (FA-A).

2 Centrifuge at 9000 *g* for 15 min to separate the HA and FA.

3 After centrifugation, the supernatant (FA) is removed and retained in vials for analysis. Then 50 mL of 0.1 *M* NaOH is added to the centrifuge bottle to redissolve the precipitate (HA). The HA may then be transferred to a vial for storage and analysis.

4 If desired, the residue of the NaOH extraction (humin) may be fractionated into HA (HA-B) and FA (FA-B) fractions. Mix the residue with 150 mL of deionized water, sonicate for 10 min with an ultrasonifier at 125 W, and then refrigerate and allow the suspension to stand for 48 h.

5 Centrifuge at 9000 *g* for 15 min and remove the supernatant.

6 Acidify the supernatant to pH 1.5 as described above.

7 Remove the precipitate (HA-B) by centrifugation and retain the supernatant (FA-B).

8 Redissolve the HA-B in 100 mL of 0.1 *M* NaOH. Retain the solution for analysis.

52.4.3 Comments

1 HA may be de-ashed by treatment with a hydrofluoric acid–HCl solution as described in the standard IHSS method (Swift 1996). However, such treatments may cause losses of nitrogenous and carbohydrate components (Schnitzer and Schuppli 1989).

2 Samples may be freeze-dried to provide dry humic materials for analysis. A variety of analyses may be performed on the dissolved humic constituents, including elemental analysis by digestion or automated combustion.

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3 The carbon content of dissolved humic fractions is commonly measured using a soluble carbon analyzer (see Chapter 48).

4 The ratio of E4:E6, which gives an indication of the molecular weight of the humic substances, may be determined by dissolving an aliquot of HA or FA (a C concentration of 34 $\mu g\ g^{-1}$ works well) in 0.05 *M* $NaHCO_3$ and measuring the ratio of the absorbances at 465 and 665 nm (Kononova 1966; Anderson et al. 1974). The IR and ^{13}C NMR spectra of the humic substances can provide important information on the chemical structure (Schnitzer and Schuppli 1989). Similarly, the FA may be passed through a column of XAD-8 resin to reduce ash content, as described in the standard IHSS method (Swift 1996).

5 Hydrolysis reactions during alkali extractions may be of concern in the study of organic S in humus. The presence of inorganic sulfate in FA extracts has led to the suggestion that organic sulfate groups are hydrolyzed to inorganic sulfate in the NaOH (Schoenau and Bettany 1987). The occurrence of such artifacts emphasizes the need for caution when interpreting the results of organic matter fractionation.

52.5 HUMUS CHARACTERIZATION OF WHOLE SOILS

Several methods are suitable for *in situ* analysis of organic matter in soils and include solid-state ^{13}C NMR spectroscopy (see Chapter 53) and pyrolysis-field ionization mass spectroscopy (Py-FIMS) (Leinweber and Schulten 1999). Hatcher et al. (2001) discusses modern analytical methods that include Py-FIMS and several others developed mainly in the biochemistry field and applicable to the study of complex biomolecules such as humic substances.

Jokic et al. (2003) studied the chemical structures of humus and the organic sulfur species present using a combination of ^{13}C NMR and a synchroton-based method, x-ray absorption near-edge structures (XANES). The method, more specifically K-edge carbon XANES, yielded information on the relative proportions of aliphatic, aromatic, and carbohydrate structures in the organic fraction of whole soils. Work with N-XANES that examined N compounds and identified both amino and heterocyclic forms of N indicates the promise of synchroton spectroscopy in studies of humus (Jokic et al. 2004).

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Chapter 53
Soil Organic Matter Analysis by Solid-State ^{13}C Nuclear Magnetic Resonance Spectroscopy

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53.1 INTRODUCTION

The use of solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy to examine the nature of soil organic matter (SOM) has gained popularity over the past two decades because it provides information regarding the chemical nature and relative quantities of carbon (C) structures (Preston 1996; Kögel-Knabner 2000; Preston 2001). The knowledge gained from solid-state ^{13}C NMR has expanded our understanding of SOM structure and is unparalleled in comparison to other techniques because it enables the nondestructive analysis of whole soil samples. Analysis of SOM by solid-state ^{13}C NMR provides information regarding the relative quantities of unsubstituted and substituted aliphatic, aromatic, phenolic, carboxylic, and carbonyl C. In addition, only small quantities of sample are needed (100–500 mg of sample depending on the type of NMR probe) to perform the analysis.

The most commonly applied solid-state ^{13}C NMR method is cross polarization with magic angle spinning (CP/MAS). The CP technique transfers the polarization from abundant ^{1}H nuclei to the less-abundant ^{13}C nuclei and enhances the ^{13}C signal by a factor of up to 4 with actual enhancement factors varying between 1 and 3. The other advantage of the CP method is that it relies on the relaxation time of ^{1}H nuclei which is considerably shorter than that of ^{13}C nuclei, reducing the time required between pulses. Therefore, CP is much faster than direct ^{13}C polarization methods (such as Bloch decay, BD) and allows one to obtain semiquantitative information about the chemistry of C in a soil within a reasonable amount of time (12–24 h). In solid-state ^{13}C NMR, samples are spun at the magic angle of 54.7° to reduce broadening due to chemical shift anisotropy (CSA), which arises from different orientations of molecules in the

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solid phase (without MAS, it is possible to observe all the signals from different molecular orientations). In addition, high-power decoupling eliminates the broadening due to dipolar interactions between ^{13}C and ^{1}H.

As NMR technology advances, low-field (100 and 200 MHz) instruments are generally being replaced with higher field (300 MHz and higher) spectrometers. Techniques differ depending on the magnetic field strength (Preston 2001; Dria et al. 2002; Smernik 2005). For example, at higher field strengths, one must use faster spinning rates to remove spinning sidebands (SSBs) that arise from CSA from the spectral window. However, CP efficiency decreases at higher spinning speeds (although BD efficiency is unaffected), and high-speed rotors have lower sample volumes. At higher spinning rates, ramped amplitude (RAMP-CP) pulse programs are often applied to compensate for CP inefficiency, although this capability may not be available on older instruments. If a higher speed probe is not available (i.e., 10–13 kHz at 300 MHz), CP/MAS with total suppression of sideband (TOSS) can be employed. However, it should be noted that the TOSS method may result in distortion of peak areas and lower signal to noise ratio (S/N) that in turn increases the experimental time.

Samples low in organic C and/or samples that are rich in iron oxides should be treated with hydrofluoric (HF) acid before ^{13}C NMR analysis (Preston et al. 1989; Skjemstad et al. 1994; Schmidt et al. 1997; Gélinas et al. 2001; Smernik and Oades 2002; Gonçalves et al. 2003; Schilling and Cooper 2004). Concentration of the organic C content through demineralization of iron-bearing paramagnetic minerals enhances the S/N and often reduces the duration of the experiment. Samples rich in carbonates should be pretreated with hydrochloric (HCl) acid to increase the relative concentration of organic C in the sample. Different concentrations of HF and HCl have been used in the past to reduce the iron content and increase the amount of organic C in a sample. Schmidt et al. (1997) did not detect a change in the organic C distribution after samples were treated with 10% (v/v) HF. Gonçalves et al. (2003) reported C losses in B horizons when using 10% (v/v) HF but did not observe any change in the distribution of C functional groups. Simpson and Hatcher (2004) used lower concentrations (0.1 *M* HCl/0.3 *M* HF) to de-ash samples to prevent any losses of organic C in the sample.

This chapter is to be used as a practical guide to accompany published works. Several review articles have summarized various aspects of methodology and theory of NMR techniques and it is highly recommended to consult related literature for a detailed account of NMR analysis of SOM. Preston (2001) reviewed NMR techniques for SOM, Dria et al. (2002) provide a brief summary of NMR theory and more detailed information on solid-state NMR can be found in Bryce et al. (2001). This chapter will describe practical aspects of preparing samples, acquiring, and processing NMR data. Pretreatment of soil samples with HF acid and HCl acid (for samples rich in carbonates) is highly recommended. This pretreatment concentrates the organic matter, which enhances the S/N of the resulting NMR spectra and removes paramagnetic minerals, such as iron, which can interfere with the acquisition of data.

53.2 SAMPLE PRETREATMENT

53.2.1 Materials

1 Analytical balance.

2 0.1 *M* HCl/0.3 *M* HF solution. *Note*: HF is highly toxic and should only be used in a fume hood. HF will also dissolve glass so only plastic or stainless steel laboratory

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ware should be used when handling HF. Gloves should be worn at all times. As a safety measure, calcium gluconate cream (HF antidote cream) should be kept on hand in the event of contact with skin.

3 250 mL, high-density plastic centrifuge tubes with leakproof lids.

4 Horizontal shaker.

5 High-speed centrifuge (capable of 4000 *g*).

6 Deionized water.

7 Freeze drier.

53.2.2 Procedure for Sample Pretreatment

1 Weigh approximately 5–10 g of air-dry sample into a 250 mL plastic centrifuge tube. *Note*: Soil samples should be first passed through a 2 mm sieve and then finely ground (<200 μm in size).

2 Add 200 mL of 0.1 *M* HCl/0.3 *M* HF solution or 0.3 *M* HF solution for samples that are carbonate-free.

3 Shake samples for 18–24 h.

4 Centrifuge samples at 4000 *g* for 20–25 min.

5 Carefully decant the supernatant and replace with fresh 200 mL of 0.1 *M* HCl/0.3 *M* HF solution or 0.3 *M* HF solution and repeat until the organic C content of the sample reaches at least 25%. *Note*: This procedure may need to be repeated a number of times for samples that are low in SOM. HF/HCl solutions should be sent for waste disposal and should not be poured down the sink.

6 Remove excess HCl/HF from the sample by rinsing the sample with deionized water. After removing the last HCl/HF solution, add deionized water, shake the sample for 4–6 h, and then centrifuge at 4000 *g* for 20–25 min. Repeat at least three times to remove excess salts. *Note*: The presence of HF/HCl can cause damage to instruments such as elemental analyzers.

7 Freeze-dry the sample and store in a sealed container for ^{13}C NMR analysis.

53.3 PROCEDURE FOR ^{13}C NMR ANALYSIS

53.3.1 Safety Procedures

A superconducting magnet is always on—do not approach the magnet (this includes from the floors above and below) if you have a pacemaker or similar device installed. Remove metal objects (e.g., keys, paper clips, tools, and penknives), wallets with magnetic strip cards, watches, computer disks, and electronic devices before approaching the magnet. The latter can be damaged, and especially with larger magnets, metal objects can turn into

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missiles, causing injury or catastrophic magnet damage (more than $100 K). Be careful not to step on any of the connecting cables.

53.3.2 Sample Preparation

Samples should be added to the rotor in small increments and packed down firmly using the packing tool provided with the instrument. Where sample quantity is less than rotor volume, either packing or leaving the sample loose may be recommended. Balanced spacers may also be used when sample size is limited (especially when spinning at high speeds). Rotor components must be firmly and completely assembled. Poor sample preparation and rotor assembly may result in difficulties with achieving stable spinning or even result in catastrophic rotor or probe failure.

53.3.3 Acquisition Parameters for CP/MAS

CP/MAS is the most common experiment used for analyzing SOM because it is faster than the direct methods discussed below. CP transfers magnetization from abundant ^{1}H nuclei to the less-abundant ^{13}C nuclei and also takes advantage of the relatively fast relaxation of protons and thus allowing the user to acquire signal intensity more quickly than with direct methods.

The following are only general guidelines for setting acquisition conditions; details of spectrometer operation vary widely, therefore consultation with the NMR spectroscopist is highly recommended. The main acquisition parameters are relaxation delay (time required for the nuclei to reequilibrate), proton 90° pulse length, CP contact time (the length of time during which magnetization is transferred from ^{1}H to ^{13}C nuclei), sweep width (SW) (range of frequencies covered in a signal pulse), and acquisition time (the length of time a given free induction decay [FID] is monitored). The recycle time (time between consecutive scans) is the sum of the acquisition time and relaxation delay.

1 *Relaxation delay*: The relaxation delay must be long enough to achieve complete spin–lattice relaxation of the protons (five times the proton spin–lattice relaxation time, T_1H note: spin–lattice relaxation occurs in the longitudinal direction (*z*-axis) corresponding to the magnetic field). This delay is approximately 4 s for glycine, 1–2 s for SOM samples high in C, such as litter and forest floor, and is often reduced to 0.4 or 0.5 s for low-C mineral soil samples. If proton spin–lattice relaxation is not complete, the S/N ratio is reduced. The other constraint is that very rapid pulsing may cause sample heating or excessive load on the high-power components, so that operators may recommend a longer relaxation time than required by the T_1H constraint.

2 *Proton 90° pulse length*: The proton 90° pulse length is typically 3–4 μs, and should be previously determined on a standard such as glycine. It is usually not determined directly, but by looking at the change in the ^{13}C CP signal as the proton pulse length is varied. The NMR signal is a null at 180° and 360° and negative at 270°. At the start of the CP pulse sequence, the proton magnetization is rotated 90° into the *x*–*y* plane, and remains locked there during CP when the ^{13}C and ^{1}H fields are matched in the rotating reference frame.

3 *Cross polarization contact time*: The CP contact time (t_c) is the time during which magnetization is transferred from protons to C. The CP process is most

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efficient for C in rigid structures with attached protons, and weakened for C remote from protons, and in structures with motion in the solid state. Therefore, CP is typically very efficient for *O*- and di-*O*-alkyl C in cellulose, and much slower for highly condensed aromatic structures, such as those produced by biomass burning. CP is also less efficient for long alkyl chains that have rotational motion and for methyl and methoxyl groups that can rotate (Alemany et al. 1983). In general, the highest overall intensity is typically found with t_c around 1 ms, but it is sometimes increased to 1.5 or 2 ms to improve the representation of aromatic C, such as those in charcoal. Simply increasing t_c will not continue to increase signal, because of the decay of the proton magnetization in the rotating frame (with a time constant of $T_{1\rho}H$ which is the spin–lattice relaxation time of protons in the rotating frame and is indicative of the rate of proton magnetization decay during contact time). The presence of paramagnetic species such as iron oxides or copper (Cu^{2+}) also reduces CP signal intensity because they reduce the $T_{1\rho}H$ of nearby protons, thus shortening the time that they can transfer magnetization. During simple CP, the proton and C power levels are constant, but with RAMP, either C or proton power may be varied to enhance CP efficiency. This largely compensates for the loss of CP efficiency with high-speed spinning.

After CP, the proton power is left on at sufficient levels to decouple the protons, and the FID signal is acquired. This is the superposition of all the frequencies in the spectrum decaying in time. It may last <5 ms for mineral soil samples, up to 20 ms for fresh litter, and several hundred milliseconds for pure compounds. Observing the FID on the screen gives an idea of the quality to be expected. A fast buildup of signal is expected from a high-C sample, while very broad signals, such as C close to paramagnetic centers and from probe background give a very short-lasting spike. The acquisition time (AQ) should be long enough to capture the FID faithfully, as cutting it off causes artifacts around the baseline and loss of resolution. Alternatively, there is no need to go beyond about twice the length of the FID, as this would simply acquire noise and add to the duty cycle of the instrument. The raw data are stored as the FID, allowing multiple processing.

4 *Sweep width*: For SOM with a chemical shift range of around 200 ppm, the SW should be at least 300 ppm, even up to 400 ppm for samples expected to give broad and weak signals. At 75 MHz for ^{13}C, this means 22,500 to 30,000 Hz. The minimum sampling rate is twice the frequency (called the Nyquist frequency), so for example, with 25,000 SW, the sampling rate is 50,000 Hz, or 20 μs/point (this is called the dwell time). For a 1 K spectrum with 1024 data points, this makes the acquisition time 20.48 ms. This gives a digital resolution of 24.4 Hz/point (sufficient for most SOM samples).

5 *Acquisition time*: For a low-C sample, the acquisition time may even be reduced to 512 points (10.24 ms), but with the sampling rate still corresponding to 1024 points. To increase digital resolution the spectrum size can be increased to 2 K or 4 K, but again, the acquisition time can be shortened if appropriate. These conditions are quite different than for solution (or even solid state) NMR of pure compounds, where the SW is minimized, and data size and acquisition time are typically much larger to optimize resolution.

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53.3.4 Direct Polarization or Bloch Decay Parameters

Direct observation experiments are sometimes preferred to analyze SOM. BD is also referred to as direct polarization (DP) or single-pulse excitation (SPE) and is a simple acquisition using a 90° ^{13}C pulse, without intensity enhancement. This technique is employed because in CP, ^{13}C nuclei are observed indirectly through magnetization transfer from nearby ^{1}H nuclei. This may underestimate C that are not in the vicinity of protons, broadened by proximity to paramagnetic species, or have some molecular mobility, and thus, direct observation provides more quantitative intensity distribution. The maximum theoretical CP enhancement is a factor of 4 (corresponding to the $^{1}H/^{13}C$ frequency ratio); although with many SOM samples it is nearer to 1.5–2.5 (Dria et al. 2002). With BD, the relaxation delay is controlled by the spin–lattice relaxation time of ^{13}C, so that delays ($5\times{}^{13}C$ T_1) are typically 100 s. The longer relaxation time for C results in less number of scans and much longer experiment times to acquire spectra with S/N ratios comparable to that obtained by CP. In addition, BD spectra generally have much lower S/N for a given number of scans, and often lower resolution. It may not even be possible to acquire a usable BD spectrum; rather than suggesting a limiting amount of C, one predictor of success is a reasonable CP spectrum after 500 scans. A typical BD acquisition is 1000 scans, requiring at least 24 h, which can be compared with a 1000 scan CP. However, the value of BD spectra lies in their quantitative reliability.

53.3.5 Dipolar Dephasing Parameters

Another very informative experiment is dipolar dephasing (DD). The delay employed in the DD experiment allows signals from protonated C to decay before data acquisition. This results in a spectrum that contains only signals from nonprotonated C and C with molecular mobility (namely, methyl group rotation and vibration (wiggling) of long-chain CH_2 groups). In the DD experiment, decoupling is turned off during a short delay, typically 45–60 μs, between CP and acquisition. With decoupling turned off, the dipolar interactions between the ^{13}C and ^{1}H nuclei cause loss of the ^{13}C signal. Just as for CP, these interactions are weaker for C that are far from protons, or have some motion in the solid state. Therefore, DD spectra have the strongest intensity for C without attached C (typically carboxyl > phenolic > aromatic) and reduced intensity for mobile C, such as methyl, methoxyl, and long-chain CH_2 groups. While DD can be used quantitatively (Hatcher 1987; Smernik and Oades 2001a) it is usually used to obtain qualitative structural information. The dephasing time should be adjusted to null the *O*-alkyl signal around 73 ppm. It should not vary much for similar samples.

53.3.6 Total Suppression of Sideband Parameters

With instruments of 300 MHz and higher, where higher spinning speeds are not available, or not suitable (e.g., a large sample volume is needed to acquire signal), TOSS can be used to eliminate SSBs. This distorts the relative areas, not only by the loss of the intensity in SSBs, but also by the intensity losses (possibly nonselective) during the complex TOSS sequence. These have not been well-studied for SOM. The SSBs appear at multiples of the spinning speed (Figure 53.1), and their severity generally increases with chemical shift, as the bonding asymmetry increases. For example, at 300 MHz and 4500 Hz MAS, TOSS is hardly needed if the aromatic and carboxyl peaks are small. However, at 400 MHz (100 MHz for ^{13}C) with 5 kHz MAS, a spectrum with high aromaticity is hardly useful without TOSS. It is particularly useful to combine TOSS with DD, where intensity is concentrated in high SSB regions, and the results are used qualitatively.

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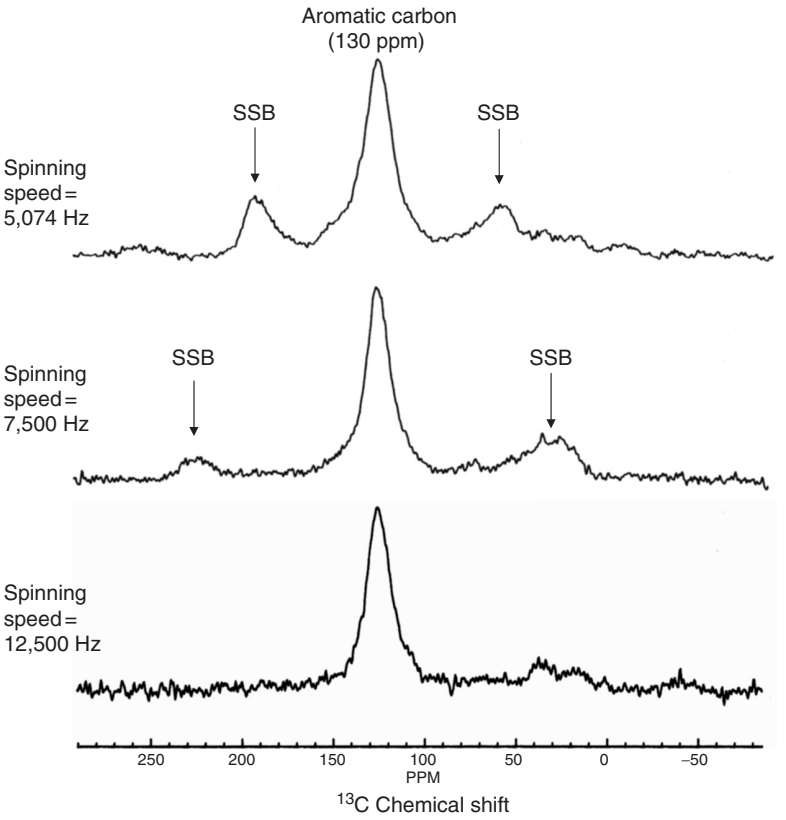

FIGURE 53.1. CP/MAS ^{13}C NMR spectra of charred biomass acquired at 75 MHz (300 MHz instrument) at different spinning speeds illustrating the presence of spinning sidebands (SSBs) at low-spinning speeds. SSBs are spun outside of the spectral window (SW) at higher spinning speeds (12,500 Hz).

53.3.7 Background Signal Considerations

The background signal from rotor and probe components is usually a problem for BD spectra, and CP spectra of samples low in C (Preston 2001; Smernik and Oades 2001b). This is usually seen as a very broad signal with a maximum of around 110–115 ppm, so that it may be interpreted as high aromaticity. Background varies with the type of probe and rotor, and may also be more severe for samples low in C and high in paramagnetic species as the latter seem to induce initial spikes in the FID. The usual approach is to run an empty rotor, and subtract the FIDs. It is not usually necessary to run a background for each sample, or to use the same rotor, although backgrounds must be run for each type of experiment. Smernik and Oades (2000a) found that a 10 s delay gave the same result as longer delays for the BD background signal, but this should be checked for the particular combination of spectrometer and probe. It may not be necessary to have the same number of scans (depending on the software used), as one of the FIDs can be scaled. However, if one wishes to compare the absolute intensity, e.g., of CP vs. BD, the same number of scans should be used for the sample and background. When running a background, it is useful to save the data at several different numbers of scans.

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53.3.8 Other Experiments and Considerations

Readers may be interested in other, more sophisticated experiments, such as spin counting to measure ^{13}C observability (Smernik and Oades 2000a,b), specific effects of paramagnetics (Preston et al. 1989; Skjemstad et al. 1994; Schmidt et al. 1997; Gélinas et al. 2001; Smernik and Oades 2002; Gonçalves et al. 2003; Schilling and Cooper 2004), proton spin relaxation editing (PSRE; Preston and Newman 1995), restoration of spectra via TCH and $T_{1\rho}H$ editing (RESTORE, Smernik et al. 2004), more detailed investigations of quantitation (Mao et al. 2000) and molecular mobility (Hu et al. 2000), and two-dimensional NMR techniques (Mao et al. 2001).

53.4 DATA PROCESSING

Several stages are required for data processing, and make a great difference to the final spectrum. It should also be noted that even if researchers cannot acquire their own data, it is very informative and useful for users to process and plot their own data, which is often done at a separate workstation. Fourier transformation converts the time–domain FID into a frequency–domain spectrum. Before this, the FID is usually modified to increase the S/N. The most common manipulation is line broadening (LB), in which the FID is multiplied by a decaying exponential, to enhance the initial portion where the S/N is higher. As a general guide, the LB (in Hz) is typically at least as large as the digital resolution (e.g., 30 Hz). The LB can be increased until it starts to cause loss of resolution or distortion in the spectrum. Sometimes, spectra may be plotted with different LB values, to enhance broader vs. sharper features as displayed in Figure 53.2. LB can be combined with other manipulations. If the AQ

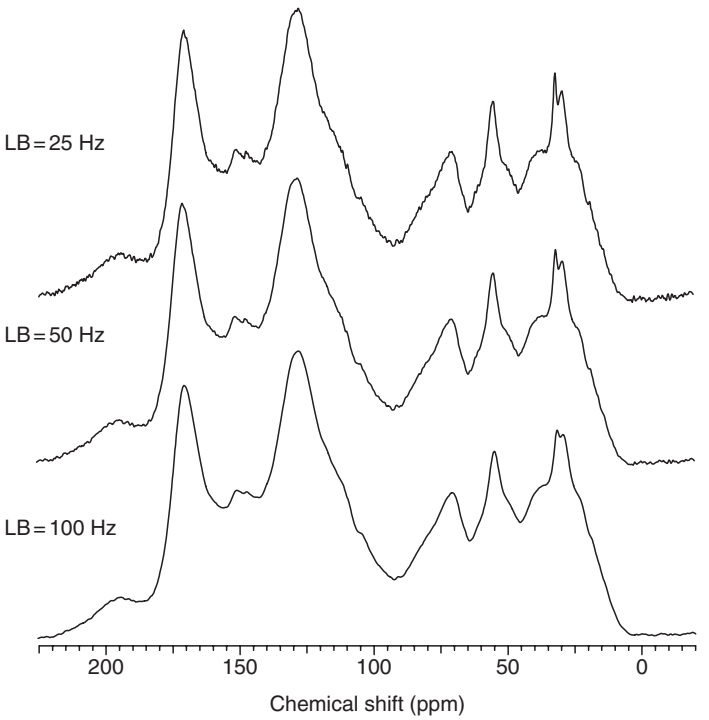

FIGURE 53.2. CP/MAS ^{13}C NMR spectra of HF/HCl-treated peat humic acid processed with different values of line broadening (LB). A loss of resolution is noticeable in the methylene C region (30–34 ppm). Data were acquired at 75 MHz (300 MHz instrument) using RAMP-CP and a MAS rate of 13 kHz.

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is much longer than the obvious FID, the last half of the FID may be set to zero. Resolution may be increased by zero-filling—doubling the data size, which interpolates points. The effect of initial spikes can be modified by setting the first two or four points to zero or removing them by left-shifting. A variety of other apodization functions are available, for which a NMR textbook should be consulted.

The purpose of phasing is to produce a pure absorption-mode spectrum, which can be difficult to do with broad features and rolling baselines. Several iterations may be required. An example of a poorly phased and a well-phased spectrum is illustrated in Figure 53.3. Often SOM spectra have a minimum around 110 ppm, and the depth of this valley can be greatly affected by phasing. After phasing, baseline correction may be needed before the spectrum can be integrated. Many NMR data analysis programs have built in automatic and manual baseline correction functions that can be employed to correct baseline irregularities before peak area integration. Integration of a ^{13}C NMR spectrum can be accomplished by using general regions, i.e., alkyl C (0–45 ppm), *O*-alkyl (45–110 ppm), aromatic and substituted aromatic (110–160 ppm), and carboxylic and carbonyl C (160–220 ppm). This approach is commonly used and area regions are typically expressed as the percentage of the total signal (as illustrated in Figure 53.4). Furthermore, chemical shifts can be used to identify specific groups of compounds typically found in SOM, such as those listed in Table 53.1. It should be noted that some NMR methods are not fully quantitative and the data should be considered as semiquantitative estimates. However, solid-state ^{13}C NMR is valuable when comparing soil samples and for understanding SOM degradation and transformation processes and in most instances can be used to make relative comparisons between samples, which are analyzed using the same NMR instrument and acquisition parameters. In addition to using general integration regions, more specific regions can be integrated that

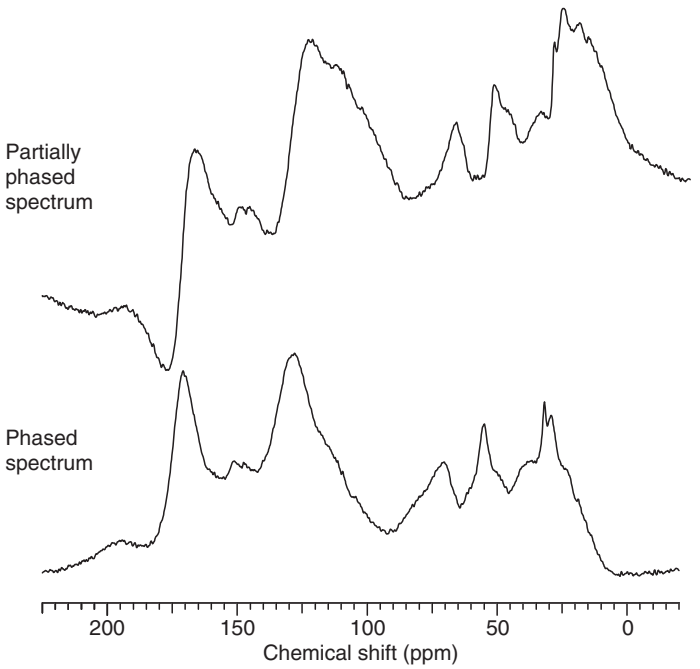

FIGURE 53.3. CP/MAS ^{13}C NMR spectra of HF/HCl-treated peat humic acid. The top spectrum has not been completely phased. When the spectrum is completely phased, both sides of the baseline will be equivalent. Data were acquired at 75 MHz (300 MHz instrument) using RAMP-CP and a MAS rate of 13 kHz.

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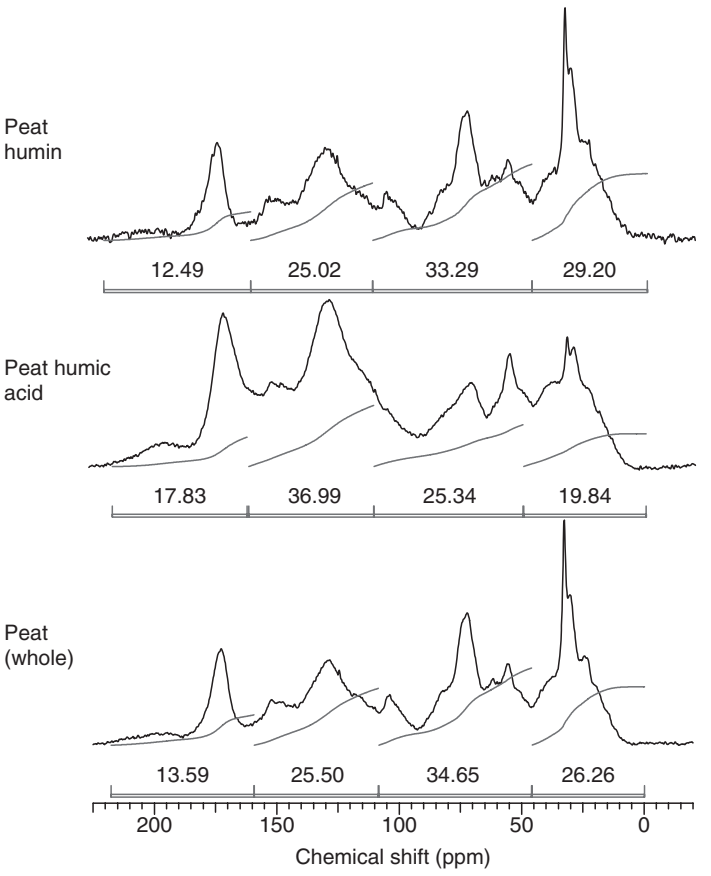

FIGURE 53.4. CP/MAS ^{13}C NMR spectra of HCl/HF-treated peat (whole), peat humic acid, and peat humin. Integrals and relative signal intensities (in percent) are displayed to demonstrate how CP/MAS ^{13}C NMR can be used to compare the relative abundance of structural groups in SOM. Data were acquired at 75 MHz (300 MHz instrument) using RAMP-CP, a MAS rate of 13 kHz and processed with a line broadening of 25 Hz.

TABLE 53.1 Typical Chemical Shift Ranges for Constituents Found in SOM

Chemical shift range (ppm)	C Structure characteristics
0–45	Unsubstituted alkyl C: Includes straight-chain methylene C (30–34 ppm) and terminal methyl groups (15 ppm). Branched methylene C is found more downfield (35–45 ppm)
45–65	Substituted alkyl C such as that found in amines (45–46 ppm) and methoxyl groups (56 ppm)
65–95	Oxygen-substituted C, ring C in carbohydrates, and C in ethers
95–110	Dioxygen-substituted aliphatic C and anomeric C in carbohydrates (105 ppm)
110–145	Aromatic C
145–160	Phenolic C
160–190	Carboxylic, amide, and ester C
190–220	Carbonyl C

Source: Adapted from Malcolm, R.L. in M.H.B. Hayes, P. MacCarthy, R.L. Malcolm, and R.S. Swift (Eds.), *Humic Substances II*, Wiley, New York, 1989, 339–372; Baldock, J.A. and Skjemstad, J.O., *Org. Geochem.*, 31, 697, 2000.

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pertain to a specific component (see Table 53.1 for examples of specific regions) or integrate from peak valley to valley.

Data interpretation can go beyond estimates of signal regions. Specific structural components of SOM can be identified based on their chemical shift. Users should keep in mind when identifying SOM structures that many structural entities will have more than one signal in addition to specific chemical shift values. For example, lignin will have both methoxy (56 ppm) and phenolic signals (140–160 ppm).

53.5 COMMENTS

Solid-state NMR methods can be extremely powerful for studying SOM structure and biogeochemistry. This technique is advantageous because it enables the nondestructive analysis of small quantities of whole soils. Samples do not need to be soluble for analysis and the analysis of whole samples reduces the formation of artifacts through extraction. The relative proportion of alkyl, *O*-alkyl, aromatic, phenolic, carboxylic, and carbonyl C can be ascertained. However, NMR instrument access may be limited thus preventing the analysis of large quantities of samples (i.e., NMR may not be used for routine analysis). Samples low in C cannot be analyzed without pretreatment due to both the low natural abundance and low inherent sensitivity of ^{13}C and consequently there are limitations to the quantitative reliability of intensity distributions especially for CP spectra (Mao et al. 2000). However, external spin counting techniques can be used to define what fraction of sample C was actually observed. Finally, the multitude of structures found in SOM may result in broad resonances, which may restrict the detail to which structural assignments can be made. However, solid-state ^{13}C NMR provides information that is complementary to other SOM methods and provides an excellent starting point for more structure-specific approaches such as multidimensional solution-state NMR spectroscopy, mass spectrometry-based analyses, and wet-chemical analysis of specific compounds.

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Chapter 54
Stable Isotopes in Soil and Environmental Research

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54.1 INTRODUCTION

Isotopes refer to elements with nuclei having the same number of protons, but differing numbers of neutrons, so that the masses of contrasting isotopes differ by one to a few neutrons. Isotopes of a specific element have the same chemical properties because they have the same number of electrons. Owing to their mass differences, however, isotopes of an element undergo chemical, biological, and physical reactions at slightly and consistently different rates, leading to isotopic fractionation whenever reactants are not exhausted. As a result, natural variations in isotopic abundance provide powerful insight into element dynamics, but fractionation by intertwined transformations can also complicate interpretations.

Isotopes of an element may be stable or radioactive. Radioactive isotopes emit radiation as they undergo radioactive decay and are transformed to new elements. One stable isotope accounts for a majority of those in most elements, and at natural abundance radioactive isotopes are far less plentiful than even the rare stable isotopes. Special reagents containing elements that are artificially enriched in one or more radioactive or stable isotopes are used in manipulative tracer studies. The distinctive isotopic composition of tracers (usually enriched in the normally rare isotope) enables element transfers and transformations to be followed in systems where otherwise it would be difficult or impossible.

Isotopic techniques to study element transfer and transformation in plants and soils were among the earliest peaceful uses of nuclear technology after 1945. These early applications typically involved adding artificially enriched radioactive (e.g., ^{32}P, ^{14}C) or stable isotopes (^{15}N), and subsequently analyzing plant tissues and soil fractions to determine the fate of the added nuclides (Noggle 1951; FAO/IAEA 1966). Similar approaches remain very useful (e.g., Frossard and Sinai 1997; Di et al. 2000; IAEA 2001). Among the earliest and most widely used stable isotopes in agroecosystems is artificially enriched ^{15}N to trace the availability and fate of fertilizer N (Norman and Werkman 1943; Hauck and Bremner 1976). Although stable isotopes tend to be more expensive to obtain and analyze than

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radioactive ones, the lack of radioactive N with a sufficiently long half-life ensured early use of ^{15}N in soil and environmental research.

In this chapter we focus on using stable isotopes, at both natural abundance and artificially enriched levels, to investigate biogeochemical cycling in soil and environmental research. We focus on stable isotopes, because (i) they include elements (e.g., ^{2}H, ^{13}C, ^{15}N, ^{18}O, ^{34}S) used to study the productivity and environmental impacts of agriculture, (ii) they have provided insight into the biogeochemical cycling of elements at both natural and artificially enriched levels, (iii) they are not subject to the safety and regulatory concerns of radioactive isotopes when applied at artificially enriched levels, and (iv) recent advances in stable isotope ratio mass spectrometry (IRMS) have improved analytical accessibility and precision. Our main emphasis will be on routine C and N isotopic analysis of common soil constituents.

54.2 NOMENCLATURE, NOTATION, STANDARDS, AND CONVERSIONS

The light elements (<40 u) with stable isotopes most widely used in soil and environmental research include H, C, N, O, and S (Table 54.1), although others have also been used (e.g., Li, B, Si, Cl, He, Ne, Ar). The abundances of the most common isotopes of each element range from 95 to 99 atom%, or more (Table 54.1). Stable isotope studies are based on measured changes in the abundance of the rarer isotopes. Larger shifts are encountered in artificial tracer studies, when reagents highly enriched in the rarer isotope (e.g., urea with 10 to 90 atom% ^{15}N) are added to the system being investigated (ranging from laboratory microcosms to field plots). These studies involve deliberate manipulation of isotopic abundance, are more amenable to carefully focused hypotheses, and target transfers and transformations over smaller temporal and spatial scales. The relatively small shifts associated with natural isotopic fractionation are usually used to study integrated effects of biogeochemical processes over large scales. Robinson (2001) compared the main features of enriched versus natural abundance ^{15}N techniques, and distinguished between using natural ^{15}N as a tracer or an integrator of N cycling processes.

TABLE 54.1 Stable Isotopes Widely Used in Soil and Environmental Research, with Corresponding Atomic Masses (u, g mol^{-1}), Abundances (Atom%)

Element	Isotope	Atomic mass (u)	Nominal abundance (atom%)
Hydrogen	^{1}H	1.007825032	99.984426
	^{2}H or D	2.014101778	0.015574
Carbon	^{12}C	12.000000000	98.894400
	^{13}C	13.003354838	1.105600
Nitrogen	^{14}N	14.003074007	99.633700
	^{15}N	15.000108970	0.366300
Oxygen	^{16}O	15.994914622	99.762060
	^{17}O	16.999131500	0.037900
	^{18}O	17.999160400	0.200400
Sulfur	^{32}S	31.972070730	95.039570
	^{33}S	32.971458540	0.748650
	^{34}S	33.967866870	4.197190
	^{36}S	35.967080809	0.014590

Source: Data after Coplen et al., Water Resources Investigations Report 01-4222, US Department of the Interior, US Geological Survey, Reston, Virginia, 2002 and references therein.

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By convention, isotope abundances are expressed as molar fractions or ratios. In artificially enriched tracer studies, abundance typically is expressed as an atom%, which is the atom fraction, F, multiplied by 100:

$$F = {}^{\text{rare}}E/{}^{\text{total}}E \approx {}^{\text{rare}}E/\left({}^{\text{abundant}}E + {}^{\text{rare}}E\right); \quad \text{for example, } {}^{13}\text{C}/\left({}^{12}\text{C} + {}^{13}\text{C}\right) \tag{54.1}$$

where ${}^{\text{rare}}E$ is the moles of the rare isotope of element E and ${}^{\text{abundant}}E$ is the moles of the most abundant isotope of the same element. Atom percent enrichment or atom percent excess (APE) is defined as the difference between sample atom% after dosing with artificially enriched tracer and the baseline or background atom%:

$$\text{APE} = \left(F_{\text{postdose}} - F_{\text{baseline}}\right) \times 100 \tag{54.2}$$

The fractional molar abundance, F, differs from the mass fraction according to differences in isotope masses (Table 54.1). The molar isotope ratio, R, is defined as follows:

$$R = {}^{\text{rare}}E/{}^{\text{abundant}}E; \quad \text{for example, } {}^{13}\text{C}/{}^{12}\text{C} \tag{54.3}$$

Thus F and R are interrelated (to close approximation; for example, contributions of radioactive ^{11}C and ^{14}C usually are negligible relative to ^{12}C and ^{13}C) as follows:

$$R = F/(1 - F) \quad \text{and} \quad F = R/(R + 1) \tag{54.4}$$

In natural abundance studies we are interested in very small variations of R, so abundances in samples of interest are expressed as differences relative to the abundance in an internationally defined standard. This method of expression is called the delta (δ) notation, and is defined as follows:

$$\delta_{\text{sample}}() = \left\{\left(R_{\text{sample}}/R_{\text{standard}}\right) - 1\right\} \times 1000 \tag{54.5}$$

with R_{sample} being the molar isotope ratio in the sample and R_{standard} being that in an international standard (Table 54.2). Thus δ values refer to isotope ratio differences, in parts per thousand, relative to a known standard. The δ value of each standard, and any sample with an identical R, is by definition 0‰. Samples that are enriched in the rare isotope relative to the standard have positive δ values, whereas samples that are depleted in the rare isotope relative to the standard have negative δ values. The absolute R of the international standards (Table 54.2) may be used to convert δ values of a sample back to the molar ratios or fractions of isotopes in the element analyzed using Equation 54.4 and Equation 54.5. To convert a δ value into atom%, the following equation may be used:

$$\text{atom}\% = 100 \times [R_{\text{standard}} \times (1 + (\delta/1000))]/[1 + R_{\text{standard}} + (R_{\text{standard}} \times \delta/1000)] \tag{54.6}$$

More information on units of measurement and their relationships can be found in Boutton (1991) and Scrimgeour and Robinson (2004).

54.3 INSTRUMENTATION

Most often, stable isotope ratios are measured using a gas source IRMS with electron impact ionization. The constituent of interest (e.g., N in whole soil, plant tissue, solute, environmental gas) must first be isolated and converted into a gas (Table 54.2) suitable for IRMS

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TABLE 54.2 Gases and Corresponding Masses Analyzed in IRMS for Stable Isotope Analysis of Light Elements, Gas Species with Interfering Masses, and Relevant International Standards with Absolute Isotope Ratios. Isotopes in Bold Refer to the Element of Interest

Element	Gas analyzed	Masses measured	Interfering masses	International standard[a]
Hydrogen	H_2	2: $^{\mathbf{1}}\mathbf{H}^{1}H$	3: $^{1}H^{1}H^{1}H$	V-SMOW
		3: $^{\mathbf{2}}\mathbf{H}^{1}H$		$2R_{\text{V-SMOW}} = 0.00015576$
Carbon	CO_2	44: $^{\mathbf{12}}\mathbf{C}^{16}O^{16}O$	45: $^{12}C^{17}O^{16}O$	V-PDB
		45: $^{\mathbf{13}}\mathbf{C}^{16}O^{16}O$		$13R_{\text{V-PDB}} = 0.0112372$
Nitrogen	N_2	28: $^{\mathbf{14}}\mathbf{N}^{14}N$		
		29: $^{\mathbf{15}}\mathbf{N}^{14}N$		Atmospheric N_2
		30: $^{\mathbf{15}}\mathbf{N}^{\mathbf{15}}\mathbf{N}$		$15R_{\text{atmN2}} = 0.0036765$
	N_2O	44: $^{\mathbf{14}}\mathbf{N}^{14}N^{16}O$	45: $^{14}N^{14}N^{17}O$	
		45: $^{\mathbf{15}}\mathbf{N}^{14}N^{16}O$		
Oxygen	CO_2	44: $^{12}C^{\mathbf{16}}\mathbf{O}^{16}O$	46: $^{13}C^{17}O^{16}O$;	V-SMOW
		46: $^{12}C^{\mathbf{18}}\mathbf{O}^{16}O$	46: $^{12}C^{17}O^{17}O$	$18R_{\text{V-SMOW}} = 0.00200520$
	CO	28: $^{12}C^{\mathbf{16}}\mathbf{O}$	30: $^{13}C^{17}O$	or
		30: $^{12}C^{\mathbf{18}}\mathbf{O}$		V-PDB
	N_2O	44: $^{14}N^{14}N^{\mathbf{16}}\mathbf{O}$	46: $^{15}N^{15}N^{16}O$;	$18R_{\text{V-PDB}} = 0.0020671$
		46: $^{14}N^{14}N^{\mathbf{18}}\mathbf{O}$	46: $^{15}N^{14}N^{17}O$	
Sulfur	SO_2	64: $^{\mathbf{32}}\mathbf{S}^{16}O^{16}O$	66: $^{32}S^{18}O^{16}O$;	V-CDT
		66: $^{\mathbf{34}}\mathbf{S}^{16}O^{16}O$	66: $^{33}S^{17}O^{16}O$;	$34R_{\text{V-CDT}} = 0.0450045$
			66: $^{32}S^{17}O^{17}O$	

[a] V-SMOW, Vienna Standard Mean Ocean Water; V-PDB, Vienna Peedee belemnite; V-CDT, Vienna Cañon Diablo troilite; *R*, molar ratio of the rare to the most abundant isotope of each element (i.e., $2R = {}^{2}H/{}^{1}H$, $13R = {}^{13}C/{}^{12}C$, $15R = {}^{15}N/{}^{14}N$, $18R = {}^{18}O/{}^{16}O$, $34R = {}^{34}S/{}^{32}S$).

analyses. For instance, to determine the isotopic composition of total soil C ($\delta^{13}C$ total C), all carbonaceous constituents (e.g., carbonates, plant residues, aromatic compounds, aliphatic compounds, and charcoal) are first oxidized to CO_2 gas. The masses analyzed by the IRMS are not 12 and 13, but 44 and 45, which represent molecules such as $^{12}C^{16}O^{16}O$ and $^{13}C^{16}O^{16}O$ (Table 54.2).

The software for most IRMS systems converts raw data (ion currents corresponding to the masses of each molecular ion) to δ values for rare isotopes of the element(s) of interest (e.g., $\delta^{13}C$), and corrects for interference by molecules with the same mass (e.g., for mass 45, $^{12}C^{17}O^{16}O$ and $^{13}C^{16}O^{16}O$ differ only in isotopic composition). For details on correcting $\delta^{13}C$ values, refer to Craig (1957), Santrock et al. (1985), or Werner and Brand (2001). Depending on the IRMS software, user intervention may be required to perform similar corrections for other gases, such as N_2O.

An IRMS comprises the following: a vacuum system, an inlet for introducing the sample gas, an ion source for ionizing the sample gas, an acceleration chamber, a magnet for separating the molecular ions (e.g., $^{12}C^{16}O^{16}O^{+}$, $^{13}C^{16}O^{16}O^{+}$, $^{12}C^{18}O^{16}O^{+}$), collectors (Faraday cups) to count the number of molecules, and a computer for instrument control, data acquisition, data storage, and data processing (converting raw counts to δ values, and sometimes computing element concentrations). The components and configuration of IRMS systems have been reviewed elsewhere (Brenna et al. 1997; de Groot 2004).

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The stable isotope ratio of an element in a sample, such as finely ground plant tissue or a vial of soil air, may be determined by continuous flow-IRMS or by dual inlet-IRMS. Although various other techniques (e.g., optical emission spectroscopy, infrared spectroscopy, quadrupole MS) have been used, here we focus on CF-IRMS. With dual inlet-IRMS, off-line procedures are used to convert sample constituents to the appropriate gas, and subsequent isotopic analysis is independent from sample preparation and conversion. With CF-IRMS, various front-end preparation devices separate, purify, and convert the samples to the appropriate gas, which is transferred (usually in a stream of He carrier gas) to the IRMS in a continuous flow. The main components that may be included in a CF-IRMS system are shown schematically in Figure 54.1.

Preparation devices are used both to convert solid or liquid samples into gases via combustion (high-temperature oxidation) or pyrolysis (thermal degradation) and to separate, purify, and concentrate gaseous or liquid samples. Elemental analyzers are used to determine the isotope ratios of C, N, and S, while pyrolysis furnaces are used for isotopes of O and H (Gehre and Strauch 2003). Trace gases, such as nitrous oxide, may be preconcentrated to increase signal strength in the IRMS. This is achieved by using an off-line technique or an online peripheral device, such as that described by Brand (1995). Gas chromatography (GC) is used to separate and analyze mixtures of gases. Depending on the compounds to be

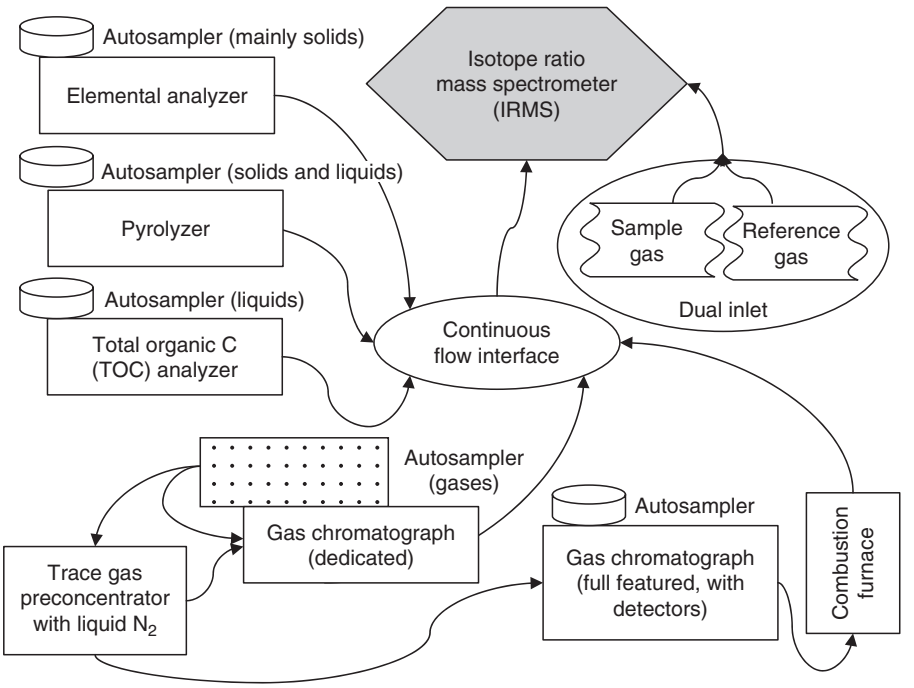

FIGURE 54.1. Schematic diagram of some common instrument configurations for analyzing stable isotopes in environmental samples by CF-IRMS using various front-end preparation devices or a dual inlet. A basic gas chromatograph (GC) to separate inorganic gases, such as CO_2, may be dedicated to CF-IRMS, whereas a full-featured GC with various detectors often is used to separate organic constituents which are oxidized to CO_2 for compound-specific ^{13}C analysis. Most CF-IRMS systems rarely include more than three preparation devices (sometimes including a dual inlet).

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analyzed, a combustion furnace may be placed after the GC column, as in compound-specific isotope analysis (CSIA) of organic samples. Detailed information on the use of GC in IRMS can be found in Hayes et al. (1989), Brand et al. (1994), Meier-Augenstein (1999a,b), Whiticar and Snowdon (1999), or Glaser (2005). For determining $\delta^{13}C$ in dissolved C species (organic and inorganic), a total organic C (TOC) analyzer for liquids may be linked via continuous flow to an IRMS (St-Jean 2003).

54.4 SAMPLE PREPARATION AND ANALYSIS

Here we focus on the isotopes of C, N, and O in soils, including solid soil samples, liquid extracts from soils, and soil gases. Teece and Fogel (2004) recently reviewed methods for collecting and storing biological materials for isotopic analyses to address ecological questions. Sample preparation for and analysis of S isotopes are described by Mayer and Krouse (2004). Methods for the isotopic composition of water in soil–plant systems are discussed by Scrimgeour (1995). Practical information on various isotope techniques and their use in soil and environmental research can be found in a recent International Atomic Energy Agency manual (IAEA 2001).

Essential to all isotope analyses is that during sample preparation all isotopes of the element in the sample are completely converted to the gas required for IRMS, and that element contamination is avoided. Incomplete conversion or contamination will likely alter the isotopic composition and compromise the results, especially in natural abundance studies (Robinson 2001).

The final isotopic composition of the sample is expressed relative to a primary international standard, such as V-PDB for $\delta^{13}C$ (see Table 54.2). CF-IRMS systems often include provisions for introducing a sample of reference gas from a pressurized cylinder. In this way, sample gases produced by various preparation devices (e.g., elemental analyzer) may be compared with a uniform aliquot of reference gas, and IRMS performance may be assessed independently. Reference materials with known δ values are also included in an analytical run to verify preparation and overall analytical quality (Coplen et al. 2002). Additional information on referencing techniques and strategies in IRMS can be found in Werner and Brand (2001).

Primary reference materials consist of well-defined minerals or chemicals (e.g., carbonate, nitrate, sucrose), and are prepared and certified by various agencies (e.g., IAEA 2004). Secondary standards, such as soil or plant material that has been calibrated against an internationally certified standard, are often used to verify routine analyses. Ideally, these secondary standards would have a similar background matrix and chemical composition as the samples (Jardine and Cunjak 2005). To this end, IRMS laboratories working with soils and plants are encouraged to develop calibrations for widely distributed soil and plant materials that have some sort of certification for total C and N contents (e.g., see http://chemsrv0.pph.univie.ac.at/wwa/homepage_ww_neu/nist.htm [validated April, 2006]). Isotope values for C and N in natural matrix materials may be available from commercial suppliers (e.g., Elemental Microanalysis Ltd., Okehampton, Devon, UK).

To analyze $\delta^{15}N$ or $\delta^{13}C$ in soils and plants by CF-IRMS, we adjust sample and standard weights so that every capsule contains a similar amount of N or C. This means that much smaller weights of pure chemical standards with high C and N contents may be intermingled with plant and soil samples. Although uncertainty in sample weight should not appreciably

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influence isotope measurements, they can influence measured N and C contents. Analytical conditions are more uniform when each capsule contains similar amounts of the element being analyzed and the background sample matrix is comparable.

54.4.1 Determination of the $\delta^{13}C$ Value for Soil Organic Matter

Inorganic C must be eliminated to analyze ^{13}C in soil organic matter, because many soils contain carbonates with greater $\delta^{13}C$ values ($\approx 0‰$ for primary carbonate) than those of organic matter. Small-scale acidification in silver capsules is useful to eliminate inorganic C after weighing and before combusting in an elemental analyzer coupled to an IRMS. This approach is widely used for marine sediments (Nieuwenhuize et al. 1994; Ryba and Burgess 2002), and may be extended to soil organic ^{13}C analysis. Since the organic C concentration is based on the preacidified sample weight, any weight change associated with acidification is immaterial. The C remaining after acidification consists of organic as well as charcoal-like materials, including those in soluble and fine particulate fractions.

Typically, between 10 and 70 mg soil is weighed into silver capsules, depending on the expected amount of soil organic C and instrument sensitivity. Greater sample weights are used when the C or N contents are low, so similar amounts of C or N per capsule are introduced to the elemental analyzer. With prior information on total C and N contents, the amount of inorganic C to be removed by acidification may be estimated as total C minus total N $\times$ 10, assuming an organic C/N ratio of $\approx$10. For example, a 50 mg soil sample containing 0.10% total N and 3.00% total C would have an expected inorganic C content of $\approx$2% (and contain 1 mg). Further assuming this 1 mg is present as $CaCO_3$, at least 28 μL 6 *M* HCl would be required to liberate the carbonate as $CO_{2(g)}$ (at least 167 μL 6 *M* HCl would be required for 50 mg pure $CaCO_3$).

A micropipette is used to dispense 50 to 80 μL of 6 *M* HCl into each capsule. The HCl is added in aliquots of 5 to 10 μL (smaller aliquots for more reactive samples) to every capsule in a tray holding 50, before adding successive aliquots. Others prefer unattended acidification whereby HCl vapor is allowed to diffuse, over several hours in an enclosed desiccator, to samples that have been weighed into capsules and moistened (Harris et al. 2001). Small-scale acidification is hampered when effervescence during the reaction of HCl with soil inorganic C causes capsules to overflow. We prefer direct addition of aqueous HCl, because the addition rate may be adjusted to contain effervescence, and samples are under close observation so that overflowing capsules are easily identified. In such rare instances, the problem sample is discarded and a new sample reacidified. To better contain samples and minimize overflowing, we use silver capsules with 8 mm diameters (wall height 11 mm), and secure 50 capsules in an acrylic holder (custom-built) for acidification and drying.

The total volume of HCl added is sufficient to react with at least 2 times the expected amount of inorganic C in the sample. So long as excess acid is added, it is more important that the entire sample is thoroughly wetted by and reacted with the HCl. For capsules containing >0.5 mg inorganic C (e.g., carbonate-rich subsoils), an intermediate drying period is used to drive off excess liquid after about 80 μL HCl has been added, and then further 10 μL aliquots are added to ensure thorough acidification (rarely more than 240 μL in all). Before the capsules are crimped closed, they are placed in a vacuum oven (approximately 16 h at 65°C and 20 kPa) to remove water and unreacted HCl. After drying, the capsules should be crimped closed and analyzed without delay or kept in a desiccator. If this is not done, calcareous samples will take up atmospheric water, because the reaction of HCl with soil carbonate produces $CaCl_2$ which is hygroscopic.

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With silver capsules, the elemental analyzer must be configured properly (adequate temperature, proper O_2 dosing) to ensure complete combustion without the exothermic reaction obtained when tin capsules are combusted (these are corroded by HCl and fail to contain acidified samples). Since the silver capsules do not fully disintegrate in the combustion tube, the ash tends to build up faster and must be removed more frequently to ensure thorough combustion. To avoid inadvertent carbonate losses, the nonacidified samples used to calibrate elemental analyses are kept separate from the acidified samples until the autosampler carousel is loaded.

54.4.2 Determination of Total Soil $\delta^{13}C$ and $\delta^{15}N$ Values

Analysis of total $\delta^{13}C$ and $\delta^{15}N$ values for whole soil is straightforward: finely ground samples (typically 5 to 50 mg) are weighed into tin capsules, and then combusted in an elemental analyzer coupled to an IRMS. Sometimes the isotopes of both elements are determined in a single run (via peak jumping whereby the IRMS is temporarily retuned from masses 28, 29, and 30 to 44, 45, and 46). But this usually involves automated dilution with He at the elemental analyzer–IRMS interface to lower effluent [CO_2] to a measurable range with other parameters optimized for N_2. Isotope ratios of C and N are often determined in separate analytical runs, but the thermal conductivity detector of the elemental analyzer can measure the concentrations of both elements in a single run. Thus the isotope ratio of one element and the concentrations of both C and N may be analyzed in a singe run without dilution or peak jumping. Total soil N concentrations (and $\delta^{15}N$), however, are best determined on nonacidified soil, because acidification may degrade analytical accuracy and precision for N (Harris et al. 2001; Ryba and Burgess 2002). When the main focus is soil organic ^{13}C, we analyze nonacidified samples for total C and N using an independent elemental analyzer, and then analyze acidified samples to determine the organic C content and the $\delta^{13}C$ value by CF-IRMS.

Baccanti et al. (1993) reviewed the main requirements to successfully implement automated flash combustion of C and N. These include proper O_2 dosing to ensure complete oxidation without excessive consumption of reduced Cu, which is used to convert any NO, NO_2, and N_2O to N_2 (essential to recover total sample N and to prevent isobaric interference by N_2O during CO_2 analyses), maintaining a leak-free autosampler to exclude atmospheric N_2 and other extraneous gases, removing ash to maintain combustion in the hot zone, and maintaining unobstructed carrier gas flow through the entire system (especially the $MgClO_4$ moisture trap). Sometimes we use smaller sample weights and/or larger O_2 doses for analyzing C and N in plant tissues (5 to 10 mg and 15 mL at 200 kPa) compared to soils (25 to 75 mg and 10 mL at 200 kPa).

Since the thermal stability of many carbonate minerals is greater than that of most organic constituents, the elemental analyzer must be configured to attain complete conversion to $CO_{2(g)}$ where carbonates are present. To analyze $\delta^{15}N$ in carbonate-rich subsoils with low N contents (i.e., total $C/N > 20$), sometimes we use soda lime to remove CO_2 from the combustion effluent, otherwise CO_2 fragmentation in the IRMS ion source produces CO ions that may interfere with determination of masses 28, 29, and 30 (Table 54.2). At narrower C/N ratios (≤ 20), N_2 and CO_2 peaks are clearly separated, and any CO produced in the source may not be problematic.

54.4.3 Determining $\delta^{15}N$ Value of Ammonium, and $\delta^{15}N$ and $\delta^{18}O$ Values of Nitrate

Various techniques have been used to determine the $\delta^{15}N$ values of ammonium (NH_4^+) and nitrate (NO_3^-). Often nitrate plus nitrite are separated and chemically reduced to NH_4^+,

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and then the procedures are the same as when NH_4^+ is the starting point. These include steam distillation, diffusion techniques, and sorption on cation-exchange resin. Diffusion techniques have been widely used in soil and environmental research, including hydrology. By increasing sample pH, aqueous NH_4^+ is converted to NH_3 gas, which diffuses to an acidic trap where it precipitates (e.g., as $(NH_4)_2SO_4$ when trapped by H_2SO_4) after drying. The precipitate is enclosed in a tin capsule, so the $\delta^{15}N$ value may be determined by CF-IRMS using an elemental analyzer. Sebilo et al. (2004) describe an ammonium diffusion technique for determining the $\delta^{15}N$ values of NH_4^+ and NO_3^-. Diffusion techniques, however, do not enable determination of the $\delta^{18}O$ value in nitrate.

Kohl et al. (1971) were among the first to test whether N isotopes can be used to identify the origin of aqueous N in surface water. Their paper sparked insightful discussions on the pros and cons of using natural ^{15}N abundance to study agricultural N dynamics. Taken alone, however, $\delta^{15}N$ values fail to distinguish among various nitrate sources, because the $\delta^{15}N$ values overlap and isotopic fractionation by various N transformations may further obscure the original source signature(s) (e.g., Kendall 1998). Clearer differentiation among nitrate sources became possible, however, after a new technique for measuring $\delta^{18}O$ values in nitrate was introduced by Amberger and Schmidt (1987). $\delta^{18}O$ values in conjunction with $\delta^{15}N$ values of nitrate have been used to assess nitrate sources and transformations in hydrological studies (e.g., Aravena et al. 1993; Durka et al. 1994; Wassenaar 1995; Chang et al. 2002; Rock and Mayer 2002, 2004), although rarely in soil–plant studies. Soil nitrate may originate from several sources, including soil organic N mineralization, inorganic fertilizers, organic amendments, and atmospheric deposition. Hence, the combined use of $\delta^{15}N$ and $\delta^{18}O$ values may provide a powerful tool to elucidate soil N sources and transformations (Högberg 1997). For instance, Mengis et al. (2001) used the dual isotope approach to trace nitrate derived from fertilizer applied to an agricultural soil in the winter when immobilization and remineralization were minimal.

Currently, the most widely used method to determine the $\delta^{18}O$ value of nitrate ($\delta^{18}O_{nitrate}$) is based on extracting aqueous NO_3^- using an anion-exchange resin, and then reacting with Ag_2O to form $AgNO_3$. Precipitated $AgNO_3$ is enclosed in silver capsules for pyrolytic conversion to CO to determine $\delta^{18}O_{nitrate}$, and in tin capsules for Dumas conversion to N_2 to determine $\delta^{15}N_{nitrate}$ via CF-IRMS with a pyrolyzer or an elemental analyzer (Silva et al. 2000). This method is not directly applicable to highly saline solutions, such as 2 *M* KCl extracts from soils, as they interfere with the ion-exchange processes. The method developed for seawater by Casciotti et al. (2002), however, can be used to determine the $\delta^{18}O$ (and $\delta^{15}N$) value of nitrate extracted from soils (Rock and Ellert 2007). The method is based on bacterial reduction of NO_3^- to nitrous oxide (N_2O) via a bacterium that lacks nitrous oxide reductase, so further reduction to N_2 does not occur. This enables determination of both the $\delta^{15}N$ and $\delta^{18}O$ values for the NO_3^- based on the measured N and O isotopic compositions of the N_2O.

54.4.4 Determination of the $\delta^{15}N$ and $\delta^{18}O$ Values of Nitrous Oxide

Trace gases, such as nitrous oxide (N_2O) and methane (CH_4), are at small concentrations ($<$10 ppmv) in most environmental air samples, so extraneous species must be eliminated and concentrations increased before isotopic analysis by CF-IRMS. To determine the $\delta^{15}N$ and $\delta^{18}O$ values of N_2O collected from soil gas samplers and aboveground air, we use a cryogenic preconcentrator (PreCon; Brand 1995) coupled to a capillary GC and an IRMS. The masses measured with the IRMS are 44, 45, and 46 (Table 54.2). Appropriate corrections must be applied to the raw δ values to account for mass interferences (e.g., Inoue and Mook 1994; Brand 1995).

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Various methods have been used to collect *in situ* soil gas and aboveground air for isotopic analysis of N_2O (e.g., Van Groenigen et al. 2005). We used horizontal soil gas sampling tubes extending into otherwise undisturbed soil to collect gas samples while avoiding mass flow along vertical samplers. Soil gas samples were collected during 2 h by connecting evacuated flasks (230 mL, stainless steel) to the gas samplers using capillaries (0.254 mm i.d. by 100 mm long). The volumes used for IRMS ranged from 230 mL when $[N_2O] \leq 4$ ppmv to 10 mL when $[N_2O] \geq 24$ ppmv (dependent upon CF-IRMS sensitivity).

In addition to analyzing the $\delta^{15}N$ and $\delta^{18}O$ values of N_2O, various spectroscopic techniques may be used to determine $\delta^{15}N$ values for specific isotopomers of N_2O ($\delta^{15}N^{\beta}$ for $^{15}N^{14}N^{16}O$ and $\delta^{15}N^{\alpha}$ for $^{14}N^{15}N^{16}O$), as the N_2O molecule has an asymmetric linear structure (Brenninkmeijer and Röckmann 1999; Toyoda and Yoshida 1999). Isotopomers (a contraction of isotope isomer) refer to molecular structures with the same numbers of each isotopic atom but differing in their positions. Beyond bulk $\delta^{15}N$ and $\delta^{18}O$ values, isotopomer determinations may help to further elucidate the relative contribution of nitrification and denitrification to soil N_2O production (e.g., Pérez 2005), although they may not provide unambiguous proof of N_2O origin (Schmidt et al. 2004). Elucidating N_2O sources is crucial if we are to effectively manage soils to reduce N_2O emissions, which often account for a majority of greenhouse gas emissions from primary agriculture.

54.5 LIGHT ISOTOPE APPLICATIONS AND CALCULATIONS

For many years the main application of stable isotope techniques in soil and environmental research centered on ^{15}N-enriched fertilizers to assess the fate of applied N, and later this was extended to probe other N transformations, including biological N fixation, denitrification, and mineralization–immobilization turnover (e.g., Högberg 1997). More recently, stable isotope applications have expanded to include a wider range of elements with isotopes at both artificially enriched and natural levels. For natural abundance studies it must be recognized that some transformations (e.g., NH_3 volatilization, denitrification) may cause significant isotope fractionation, whereas others (e.g., N fixation, leaching) cause almost none. Enrichment or fractionation factors for various N cycling processes can be found in Robinson (2001) and Bedard-Haughn et al. (2003). Another trend has been greater recognition of soil as an ecosystem component, so that in addition to transformations within soils, stable isotope techniques have been used to investigate transfers to other ecosystem components (plants, animals, water, and air). Several books covering basic principles and a range of applications are available (Coleman and Fry 1991; Lajtha and Michener 1994; Sala et al. 2000; Unkovich et al. 2001; de Groot 2004; Fry 2006; Sharp 2007). Here we focus on only three common applications.

54.5.1 Vegetation-Induced Shifts in $\delta^{13}C$ Values of Soil Organic Matter

Among the most common uses of natural ^{13}C abundance in soil research is estimating the proportion of soil organic matter derived from isotopically distinct inputs (Balesdent and Mariotti 1996). As a first approximation, ^{13}C fractionation during decomposition is assumed negligible, so the $\delta^{13}C$ values of soil C inputs and the soil organic matter that accumulates are assumed to be the same (i.e., isotopic equilibrium between inputs and the organic matter pool). If ^{13}C abundance within the inputs changes, the extent to which the inputs are retained as soil organic matter or decomposed back to CO_2 may be inferred from changes in ^{13}C abundance within the organic matter pool.

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The most common cause of a change in ^{13}C abundance is a shift in the proportional contributions to soil C inputs by plants using the C3 or C4 photosynthetic pathways. Owing largely to differences in the affinity of the initial carboxylating enzyme for CO_2 (rubisco for the C3 pathway and PEP carboxylase for the C4 pathway), C3 plants discriminate more strongly against atmospheric $^{13}CO_2$ than do C4 plants. Consequently, the $\delta^{13}C$ values of C3 plants typically range from $-30‰$ to $-25‰$, whereas those of C4 plants typically range from $-13‰$ to $-15‰$.

Vegetation-induced shifts in plant ^{13}C inputs occur when native vegetation (C3 forest, C3 prairie, C4 prairie or savanna) is cleared and the land is cropped under vegetation with the contrasting photosynthetic pathway. In such cases, elementary isotope mixing models are used to estimate the proportions of soil organic C derived from each type of vegetation (i.e., that persisting from the original vegetation, and that from the more recent, isotopically distinct vegetation; Figure 54.2). With the approximation that δ values are linearly related to F (i.e., atom fraction), the slope of the mixing line is $1/(\delta_{C4} - \delta_{C3})$ and the intercept is $\delta_{C3}/(\delta_{C4} - \delta_{C3})$, where δ_{C3} is the delta value for C3 vegetation (e.g., $-28‰$) and δ_{C4} is that for C4 vegetation (e.g., $-14‰$). From the slope of the mixing line (Figure 54.2), it is often assumed that each $1‰$ increase in the $\delta^{13}C$ value of soil organic matter roughly represents a 7% increase in amount derived from C4 vegetation.

Isotopic partitioning of soil organic matter between that derived from isotopically distinct soil C inputs (p_{C4}; Figure 54.2, y-axis) and that originally present $(1 - p_{C4})$ provides useful information on C cycling. Changes in p_{C4} through time indicate the kinetics of recent C accumulation and original C persistence (Balesdent and Mariotti 1996). From the quantities of soil organic C (expressed as Mg C ha^{-1} to a specified soil depth or mass after bulk density corrections—see Chapter 3) and their $\delta^{13}C$ values, the quantities of original soil organic C persisting at various times after the shift in plant ^{13}C inputs are calculated as the product of measured soil organic C and $(1 - p_{C4})$. In the simplest instance, a first-order decay model may be fitted to original C persisting at successive times ($C_t = C_0 e^{-kt}$, where C_t is the quantity of original C at time t, C_0 is that just before the ^{13}C shift occurred, and k is the first-order decay coefficient). In turn, k, the half-life $(\ln 2/k)$, and/or the turnover time $(1/k)$ of

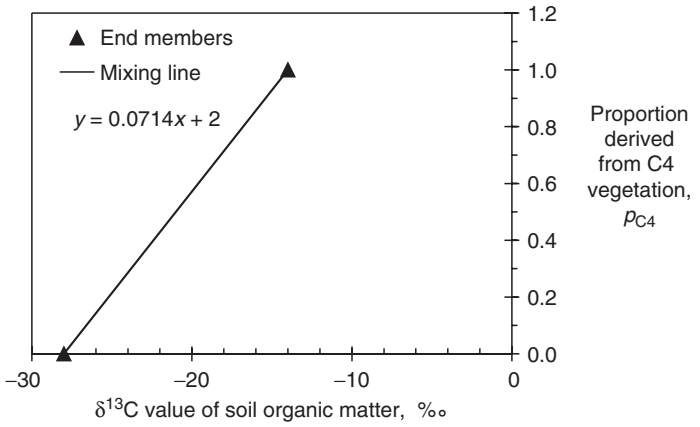

FIGURE 54.2. Elementary isotope mixing model with two end members used to estimate the proportions of soil organic matter derived from C3 and from C4 vegetation.

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the original soil organic matter may be estimated as $k = (\ln C_0 - \ln C_t)/t$. Amundson and Baisden (2000) discuss more detailed models for isotopes of soil organic matter.

Variations on the isotopic partitioning approach described above have been used in a wide range of settings. Comparisons have been based on soil samples collected at a single time from carefully paired sites including an unchanged reference site and one or more sites where the shift in ^{13}C inputs had occurred in previous years (i.e., temporal series replaced by relevant comparisons in space). Often the isotopic shift is smaller than the roughly 14‰ difference between C3 and C4 vegetation, and there is a corresponding decline in the resolution of soil organic matter sources. This approach is based on many assumptions including homogeneity of plant ^{13}C inputs, uniformity of ^{13}C abundance in organic matter among contrasting soil layers and fractions, and changes associated with decomposition. For example, $\delta^{13}C$ values of plant C (especially C3 plants) often vary among seasons, water availability, salinity, and N fertilization (Jenkinson et al. 1995; Shaheen and Hood-Nowotny 2005). Fractionation during biosynthesis also culminates in nonuniform ^{13}C distribution among various chemical constituents (Hayes 2001). Lignins and lipids often are depleted by 3‰ to 6‰ compared to carbohydrates, and these decay at different rates. Balesdent and Mariotti (1996) proposed that over the short term (2 to 10 y) there may be minimal fractionation during decay, because increases in residual ^{13}C remaining after detrital respiration are offset by the slower decay of ^{13}C-depleted lignin. In the long term, however, the effect of detrital recycling dominates, and decomposition tends to increase the $\delta^{13}C$ value of remaining organic matter. Thus $\delta^{13}C$ values of organic matter within a soil profile typically increase (by ≈1‰ to 3‰) from surface soils dominated by more recent plant C inputs to subsurface layers containing highly decomposed and persistent materials (Boutton 1991; Accoe et al. 2002).

In theory, the proportional contributions of $n + 1$ distinct sources may be calculated from the use of n different isotopic tracers (e.g., $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, $\delta^{34}S$). In practice, however, the isotopic compositions of the sources are uncertain and the number of potential sources may exceed by more than one the number of usable isotopic tracers. Often it is assumed that variations in the isotopic compositions of the sources (e.g., C3 and C4 vegetation) are negligible relative to those of the pool (e.g., soil organic C) to be partitioned. Phillips and coworkers (Phillips and Gregg 2001, 2003; Phillips et al. 2005) discuss uncertainties in source partitioning, and for systems with too many sources ($>n+1$) they evaluate the influence of source aggregation and present algorithms to calculate the distribution of feasible solutions (IsoError and IsoSource software were available, at the time of writing, from http://www.epa.gov/wed/pages/models.htm).

54.5.2 Fate of ^{15}N-Enriched Fertilizer

Many comprehensive reviews consider the use of field-applied ^{15}N to investigate N cycling in terrestrial ecosystems (e.g., Hauck and Bremner 1976; Di et al. 2000; Bedard-Haughn et al. 2003). The fundamental approach to partition some N pool (e.g., plant N, soil organic N, soil inorganic *N*, volatilized N) between natural N present originally and added ^{15}N-enriched fertilizer is analogous to partitioning soil organic matter between C3 and C4 vegetation (see Section 54.5.1). In enriched experiments, δ values are replaced by APE, and since APE of natural N is zero, the mixing line describing the proportion derived from the enriched source ($p_{\text{enriched\_source}}$) extends from the origin and the slope is the inverse of the APE for the enriched source (i.e., $p_{\text{enriched\_source}} = \text{APE}_{\text{sample}} \times 1/\text{APE}_{\text{enriched\_source}}$). The proportion of the enriched source found in plant dry matter produced during the growing season commonly is used to estimate fertilizer use efficiency (IAEA 2001). First, total plant N yield per unit

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area is estimated by harvesting and tissue analysis, then the product of this and $p_{\text{enriched\_source}}$ estimates the plant N derived from fertilizer per unit area. Finally, that number is divided by the fertilizer application rate to estimate the N fertilizer use efficiency.

A wide range of more sophisticated approaches to investigate N cycling using ^{15}N-enriched fertilizers have been developed. Stark (2000) classifies these into tracer techniques, isotope dilution techniques, and isotope models solved numerically. Estimating fertilizer use efficiency as described in the previous paragraph would fall under tracer techniques where the goal is to assess element transfer from one pool (e.g., applied fertilizer N) to another (plant N), and the advantages, assumptions, and pitfalls have been reviewed (e.g., Hauck and Bremner 1976; Stark 2000). In particular, interpretations may be confounded by N pool substitution when the N in various soil pools is replaced by the applied ^{15}N-enriched fertilizer during immobilization–mineralization turnover (Jansson and Persson 1982; Jenkinson et al. 1985). The ^{15}N-enriched fertilizer is immobilized in place of unlabeled N, so that soil inorganic N (immediately available for plant uptake) contains less ^{15}N than there would have been without N pool substitution. Since ^{15}N-enriched reagents are expensive, only small amounts are applied in most studies, and often lateral dispersion is restricted by some form of microplot to help attain uniform isotope distribution and representative sampling (e.g., Follett 2001).

Isotope dilution usually refers to techniques based on labeling a pool with known amount of isotope, and then measuring the size and isotopic abundance of the pool after a predetermined equilibration period. The extent to which the isotope becomes diluted reflects the size of the entire pool accessible to the tracer during the equilibration period (Di et al. 2000). Since isotope mass is conserved, isotope dilution measures the entire equilibrating pool without separating it from the soil. Kirkham and Bartholomew (1954, 1955) developed a series of equations to describe the exchange and subsequent transformations of enriched ^{15}N added to soil, and these equations form the basis for much recent work on deciphering gross rates of N mineralization and immobilization.

54.5.3 Estimating the δ^{15}N Value of an Unknown Source

In some instances, natural isotopic abundance may be used to identify an unknown source and its contribution to a particular biogeochemical pool. Again, users must be aware of the potential for isotopic fractionation during transfers and transformations (especially of soil N), which may complicate the interpretations (Shearer and Kohl 1993).

Consider plant foliage in which the δ^{15}N value of total N ($\delta^{15}N_{\text{foliage}}$) decreases while the concentration ([foliage N]) increases with decreasing distance from an intensive livestock operation (Figure 54.3). The foliage is from standardized plants uniformly grown in the same potting soil, and the source N responsible for increasing [foliage N] is hypothesized to be ammonia emitted from an intensive livestock operation. Changes in the δ^{15}N value of foliage with increasing N content suggest that the source N is isotopically distinct, and that its δ^{15}N value might be estimated. A plot of the measured $\delta^{15}N_{\text{foliage}}$ values versus the inverse of [foliage N] could possibly yield a straight line (Figure 54.3) with an equation in the form of:

$$\delta^{15}N_{\text{foliage}} = \text{slope} \times (1/[\text{foliage N}]) + \text{intercept} \tag{54.7}$$

The intercept of this line would estimate the δ^{15}N value of the unknown source. If the δ values represent simple mixing, a straight line will be observed between the δ values and the

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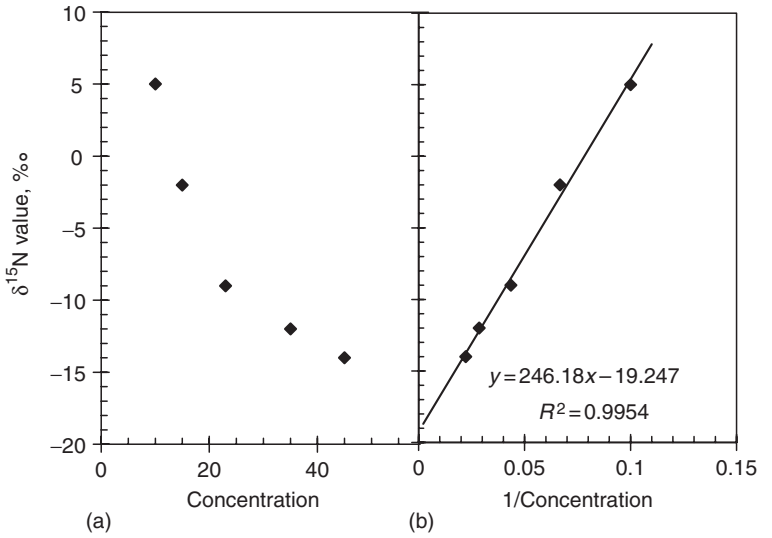

FIGURE 54.3. Hypothetical change in the $\delta^{15}N$ value of potted plant foliage versus (a) total N concentration (arbitrary units) and (b) the reciprocal of total N concentration. The *y*-intercept in panel (b) estimates the $\delta^{15}N$ value of the N source responsible for increasing the total N concentrations and concomitantly decreasing the $\delta^{15}N$ values of the foliage.

inverse of concentration. If, however, isotope fractionation occurs as element concentration increases, the line will curve (Kendall and Caldwell 1998). In turn, knowing the $\delta^{15}N$ values of the N source and of total plant foliage N without inputs from the N source, one can estimate the relative contributions of source N over distance from the livestock operation, as described for partitioning in Section 54.5.1. Similarly, Shearer and Kohl (1993) suggested: $\%N_{df\_unknown} = (\delta^{15}N_{known} - \delta^{15}N_{measured})/(\delta^{15}N_{known} - \delta^{15}N_{unknown})$ where $\%N_{df\_unknown}$ is the percentage N derived from an unknown source with a delta value of $\delta^{15}N_{unknown}$ (e.g., ammonia emitted from livestock operations), $\delta^{15}N_{known}$ is the delta value for the known source (e.g., background soil N), and $\delta^{15}N_{measured}$ is the measured delta value (e.g., for a plant or soil impacted by unknown N inputs).

Similar approaches are widely used in studies of ecosystem respiration. In these studies, the $\delta^{13}C$ values for soil-respired CO_2 may be estimated from plots of the δ values versus the reciprocal of $[CO_2]$ for a series of air samples collected when concentrations are increasing (Keeling 1958, 1961; Flanagan and Ehleringer 1998). Corrections for isotopic fractionation during CO_2 diffusion from the soil may be required.

54.6 COMMENTS ON THE USE OF STABLE ISOTOPES IN ENVIRONMENTAL RESEARCH

Advances in CF-IRMS instrumentation for light elements have improved the accessibility of stable isotope analyses to researchers. Automation has greatly increased analytical throughput, and proper implementation of CF-IRMS has decreased errors associated with sample preparation. For much soil work, field and sampling variability in isotope composition likely exceeds analytical variability. Data are easily misinterpreted if influential sources, transformations, and isotope fractionations are neglected. Consequently, investigators are

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encouraged to consider the zeroth rule of O'Leary et al. (1992): ''be sceptical of effects smaller than 1‰.'' While some differences smaller than this may be real and significant, the inherent variability of soils and the heterogeneous nature of soil organic matter may obscure small isotope effects.

Many creative techniques are available for applying isotope analyses to probe biogeochemical cycling of light elements (e.g., N, C, S) and soil ecological processes with greater clarity and specificity (e.g., Staddon 2004). The techniques are best applied, however, to well-designed studies (e.g., Pennock 2004) that are fully characterized using the best available, nonisotopic methods. Stable isotope techniques are most powerful when established on a solid foundation of more conventional analyses (e.g., robust concentration data to estimate biogeochemical fluxes and pools). Thus investigators are encouraged to heed what Kendall and Caldwell (1998) refer to as Fretwell's law: ''Warning! Stable isotope data may cause severe and contagious stomach upset if taken alone. To prevent upsetting reviewers' stomachs and your own, take stable isotope data with a healthy dose of other hydrologic, geologic, and geochemical information. Then, you will find stable isotope data very beneficial.''

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VI. SOIL PHYSICAL ANALYSES

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Chapter 55
Particle Size Distribution

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55.1 INTRODUCTION

Soil textural classes (e.g., loam, clay loam, sandy loam) are estimated in terms of size and distribution of primary particles by sieve and sedimentation analysis. Field estimates of texture are confirmed by laboratory analyses of selected samples. Particle size analysis (PSA) is a measurement of particle size distribution. The Canadian Soil Information System glossary defines particle size distribution as the amounts of the various soil separates in a soil sample, usually expressed as weight percentages (Gregorich et al. 2001).

According to Gee and Or (2002) two major features of PSA are the destruction or dispersion of soil aggregates into distinct units by chemical, mechanical, or ultrasonic means, and the further separation of these particles into size classes by sieving and sedimentation. The proportions are calculated from the relative weights of the particles within defined size classes. The primary particle size classes used in this chapter are defined in the Canadian System of Soil Classification (CSSC) (Soil Classification Working Group 1998) and are given in Table 55.1. The limits of soil particle size classes differ in various commonly used systems (Figure 55.1). The CSSC, the United States Department of Agriculture (USDA), and the International Society of Soil Science (ISSS) systems are used for soil survey data. The American Association of State Highway Officials (AASHO) and the Unified Systems (American Society for Testing and Materials [ASTM]) are used for engineering purposes.

Two common methods of PSA are the pipette and hydrometer methods. In both, the coarser fractions are measured by sieving, and the finer from sedimentation rates based on Stokes' law. Stokes' law states that the amount that a particle sinks depends upon the density of the particle, i.e., denser (larger) particles sink more than less dense (smaller) particles when suspended in a liquid. Sheldrick and Wang (1993) indicate that Stokes' law describes small spherical particles of density, PS, and diameter, D, settling through a liquid of density, PL, and viscosity, n, at a rate of

$$v = D^2 g(\mathrm{PS} - \mathrm{PL})/18n \tag{55.1}$$

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TABLE 55.1 Limits of Particle Size Classes Defined in the Canadian System of Soil Classification

Particle size	Diameter (mm)
Very coarse sand	2.0–1.0
Coarse sand	1.0–0.5
Medium sand	0.5–0.25
Fine sand	0.25–0.10
Very fine sand	0.10–0.05
Silt	0.05–0.002
Clay	≤0.002
Fine clay	≤0.0002

Source: Soil Classification Working Group, *The Canadian System of Soil Classification*, Agriculture and Agri-Food Canada Publication 1646 (Revised), Ottawa, Ontario, Canada, 1998.

where g is acceleration due to gravity. It is important to know that all sedimentation tables used for particle size distribution analysis, such as Table 55.2, are on the basis of the following assumptions: (1) soil particles are spherical (most silicate clay particles, in fact, are platy); (2) PS = 2.65 or 2.60 Mg m^{-3} (density could vary from 2.0 to 3.2 Mg m^{-3}); and (3) temperature of H_2O is constant throughout sedimentation (it is important to use H_2O at room temperature and to operate in a temperature-controlled room). It is also assumed that the settling of soil particles in a cylinder is not influenced by other particles or by the cylinder wall.

55.2 PIPETTE METHOD

The following pipette method (Soil Conservation Service 1984) was adapted from the US Soil Survey, Lincoln, NE, and reduces the time required to do the analysis by replacing the numerous centrifuge washing steps with a filter candle system. The data determined using the filter candle system corresponds well with the data from the standard pipette method and centrifuge washing (Sheldrick and Wang 1987).

If centrifuge washing is preferred, the soil samples, after pretreatments, can be washed in 250 mL centrifuge bottles with approximately 50 mL H_2O and centrifuged for 10 min at 500 g. Repeat the washing procedure three times and test for salts as described in step 2 in Removable of Soluble Salts, p. 718.

55.2.1 Material and Reagents

1 Fleakers—300 mL plus plastic caps

2 Ceramic filter candles

3 Shakers

   a. End-over-end (40–60 rpm)

   b. Sieve shaker (500 oscillations per min)

4 Cylinder—soil suspension (1205 mL) marked at 1000 mL

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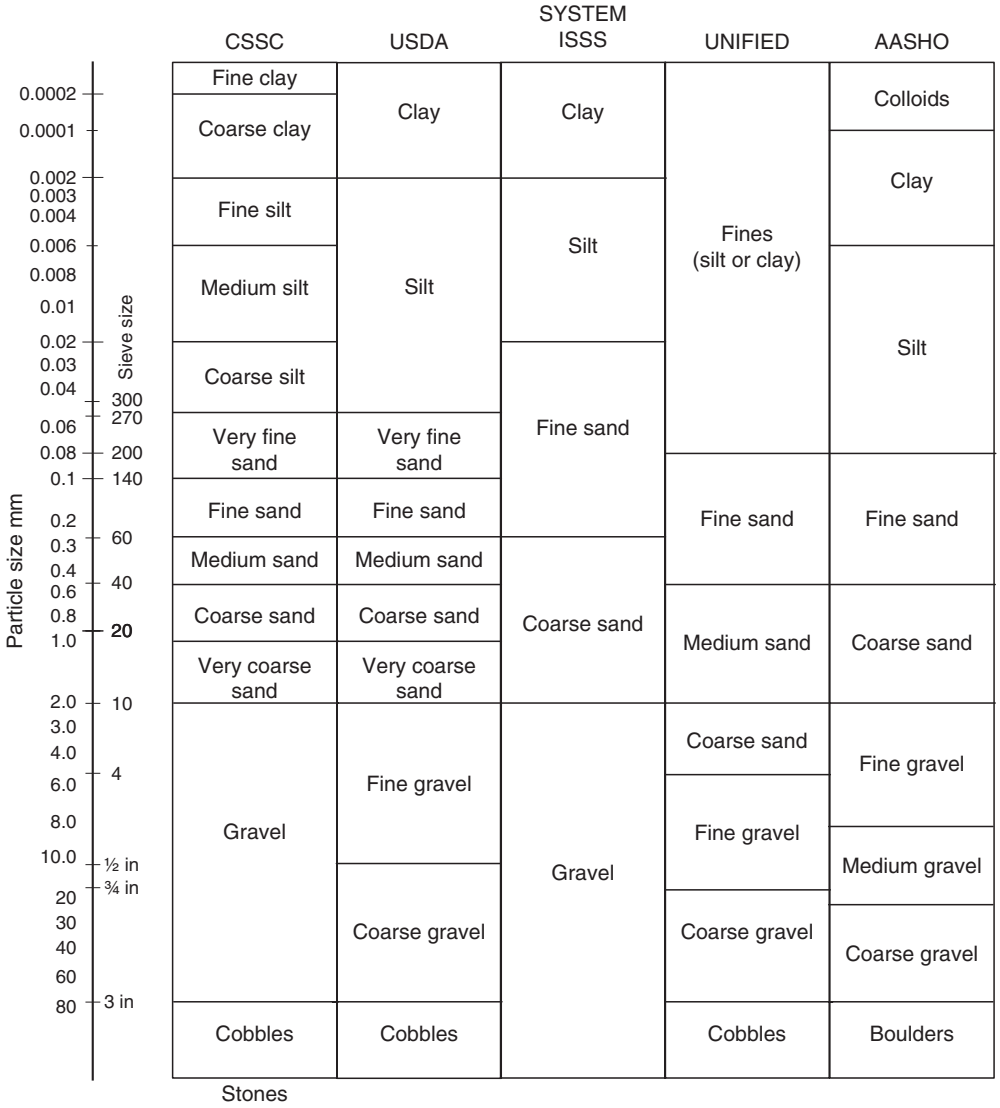

FIGURE 55.1. A comparison of particle size limits in five systems of particle size. (Adapted from Sheldrick and Wang, in M.R. Carter (Ed.), *Soil Sampling and Methods of Analysis*, Canadian Society of Soil Science, Lewis Publishers, Boca Raton, Florida, 1993. Gee and Or, in J.H. Dane and G.C. Topp (Eds), *Methods of Soil Analysis, Part 4—Physical Methods*, Soil Science Society of America, Madison, Wisconsin, 2002.)

5 Racks:

a. Custom-built frame to hold four motor-driven stirrers equipped with propeller-type stirrer and Teflon® guard

b. Shaw pipette rack modified to hold four 25 mL Lowry pipettes

c. Custom-built wood frame to support fleakers, filter candles, and vacuum system

6 Styrofoam pipe insulating cover

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TABLE 55.2 Settling Depths for Specific Times and Temperatures for Particle Size = 2 μm

	Time (h)			
	4.5	**5**	**5.5**	**6.5**
Temperature (°C)	**Depth (cm)**			
20	5.79	6.44	7.08	8.37
20.3	5.81	6.48	7.13	8.43
20.5	5.86	6.52	7.17	8.47
20.7	5.89	6.55	7.2	8.51
21	5.93	6.59	7.25	8.57
21.3	5.97	6.64	7.3	8.63
21.5	6.01	6.68	7.33	8.68
21.7	6.04	6.72	7.39	8.73
22	6.09	6.75	7.43	8.78
22.3	6.13	6.8	7.49	8.85
22.5	6.15	6.83	7.51	8.88
22.7	6.18	6.86	7.55	8.92
23	6.22	6.91	7.6	8.98
23.3	6.27	6.96	7.66	9.05
23.5	6.29	6.98	7.68	9.08
23.7	6.33	7.04	7.74	9.15
24	6.37	7.08	7.78	9.2
24.3	6.4	7.12	7.83	9.25
24.5	6.43	7.15	7.86	9.29
24.7	6.45	7.18	7.89	9.33
25	6.51	7.24	7.96	9.41
25.3	6.56	7.28	8.01	9.47
25.5	6.58	7.31	8.04	9.5
25.7	6.61	7.35	8.08	9.55
26	6.66	7.4	8.14	9.62
26.3	6.69	7.44	8.18	9.67
26.5	6.72	7.47	8.22	9.72
26.7	6.76	7.51	8.26	9.76
27	6.81	7.56	8.32	9.83
27.3	6.85	7.61	8.37	9.89
27.5	6.87	7.64	8.4	9.93
27.7	6.91	7.68	8.44	9.98
28	6.97	7.74	8.51	10.06
28.3	7.01	7.79	8.57	10.13
28.5	7.04	7.82	8.61	10.17
28.7	7.07	7.86	8.65	10.22
29	7.12	7.91	8.7	10.28
29.3	7.16	7.95	8.75	10.34
29.5	7.19	7.99	8.79	10.39
29.7	7.22	8.02	8.82	10.43
30	7.27	8.08	8.88	10.5

Source: McKeague, J.A. (Ed.), in *Manual on Soil Sampling and Analysis*, 2nd ed., Canadian Society of Soil Science Ottawa, Ontario, Canada, 1978.

7 100 mL beakers or wide-mouth glass pill bottles

8 Balance (0.1-mg sensitivity)

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9 Sieves:

a. 300-mesh, 15 cm

b. Set of sieves, brass, and 6.3 cm diameter. U.S. series or Tyler screen scale equipment designations as follows:

Opening (mm)	U.S. No.	Tyler mesh size
1.00	18	16
0.50	35	32
0.25	60	60
0.105	140	150
0.047	300	300

10 Hydrogen peroxide (30% or 50%)

11 Hydrochloric acid, 1 *M*

12 Citrate–bicarbonate buffer. Prepare a 0.3 *M* solution of sodium citrate (88.4 g L^{-1}) and add 125 mL of 1 *M* sodium bicarbonate (84 g L^{-1}) to each liter of citrate solution

13 Sodium hydrosulfite (dithionite)

14 Saturated sodium chloride solution

15 A solution of sodium metaphosphate with enough sodium carbonate to bring the pH to 10 ($NaPO_3$), 35.7 g L^{-1} + Na_2CO_3 7.9 g L^{-1} is suitable

55.2.2 Procedure for Pretreatments

Removal of Carbonates

1 Weigh 10 g of 2-mm air-dried soil into a 300 mL fleaker (tared to 1 mg). If the sample appears to be sandy, weigh a larger sample (e.g., 30 g).

2 Add 50 mL of water, mix, and add 1 *M* HCl slowly until pH reaches between 3.5 and 4.0 and remains there for 10 min. Stronger HCl can be used to avoid having a large volume of solution in soils high in carbonate content. Soils requiring a large amount of HCl to adjust the pH are washed several times with water to remove excess acid by using the filter candle system.

Removal of Organic Matter

1 Add 10 mL of hydrogen peroxide (H_2O_2, 30% or 50%) to the fleakers cover and allow to stand. If a violent reaction occurs, repeat cold H_2O_2 treatment until no more frothing occurs.

2 When frothing subsides, heat contents of fleakers to 90°C. Continue adding H_2O_2 and continue heating until most of the organic matter is removed (as observed by the color and rate of reaction of the sample).

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3 Rinse down the sides of the reaction vessel occasionally. Continue heating the sample for about 45 min after the final addition of H_2O_2 to remove excess H_2O_2.

Note: It may be necessary to transfer samples containing high amounts of organic matter (>5%) to large beakers (e.g., 100 mL tall). If excessive frothing occurs, cool the container either with cold water or by the addition of methyl alcohol to avoid sample loss.

Removal of Soluble Salts

1 Place the fleakers in a rack and filter the remaining peroxide and water off from step 3 to remove organic matter using a filter candle system.

2 Add 150 mL of water in a jet strong enough to stir the sample, and filter the suspension through the filter candle system. Five such washings and filterings are usually enough, except for soils containing large amounts of coarse gypsum. To test for salts, check with silver nitrate ($AgNO_3$) for Cl^- and barium chloride for SO_4^{-2}.

3 Remove soil adhering to the filter candle by applying gentle back pressure and using a rubber-tipped finger to loosen any remaining material.

Note: If iron oxides are to be removed do not complete step 4 at this time.

4 Place the sample in an oven at 105°C overnight, cool in a desiccator, and weigh to the nearest milligram. Use the weight of the oven-dried treated sample as the base weight for calculating the percentages of the various particle size fractions.

Removal of Iron Oxides (Optional)

1 Add 150 mL of citrate–bicarbonate buffer to the samples in the fleakers. Stir and add 3 g of sodium hydrosulfite ($Na_2S_2O_4$) gradually, as some samples may froth.

2 Place fleakers in a water bath at 80°C and stir intermittently for 20 min.

3 Remove fleakers from the bath, place in the holding rack, and filter the suspension through the filter candle system. If the sample remains a brownish color, repeat steps 1 to 3 inclusively. If the samples are completely gray (gleyed) proceed to step 4.

4 Wash five times with a jet of water strong enough to stir the sample and filter the suspension through the filter candle system.

5 To determine the oven-dry weight for calculations, repeat step 4 to remove soluble salts.

55.2.3 Procedure for Particle Size Separation

Dispersion of Sample

1 Add 10 mL of sodium metaphosphate dispersing agent to the fleakers containing the oven-dried treated samples. Add distilled water to make the volume up to 200 mL.

2 Stopper tightly and shake end-over-end (50–60 rpm) overnight.

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Separation of Sand Fractions

1 Pour the suspensions through a 300-mesh (47 μm) sieve into a sedimentation cylinder marked at 1000 mL. Place the 300-mesh sieve (14 cm diameter) in a large funnel held above the 1205 mL cylinder by a retort stand.

2 Wash the sand retained on the sieve thoroughly with a fine jet of water and collect the washings in the cylinder until the volume in the cylinder is about 950 mL. Remove the sieve and add water to make the volume up to 1000 mL.

3 Transfer the sand to a 100 mL beaker and oven-dry at 105°C. Weigh the sand and record the weight at this time if only total sand is determined. Otherwise proceed with sand fractionation.

4 Transfer the dried sand to a set of sieves (6 cm diameter) arranged as follows from top to bottom: 1.0 mm (18-mesh), 0.5 mm (30-mesh), 0.25 mm (60-mesh), 0.105 mm (140-mesh), 0.047 (300-mesh), and collecting pan. Pour the sand on to the top sieve, put cover in place, and shake the sieves on a sieve shaker. The time of shaking depends on the type of shaker and the volume of the sand in the sample (usually 5 to 10 min is sufficient). Weigh each sand fraction and record the weight.

Determination of Clay (0–2 μm)

1 Before placing the cylinder in a sedimentation room (vibration-free area equipped with a Shaw pipette rack), stir the material in the sedimentation cylinders for 4 min with a motor-driven stirrer (8 min if suspension has stood for longer than 16 h).

2 Remove from stirrer and place a length of Styrofoam pipe-insulating cover over the sedimentation cylinder. Stir the suspension for 30 s with a hand stirrer using an up-and-down motion. Note the time at the completion of stirring.

3 Sample the 2 μm fraction after a predetermined settling time (usually 4.5 to 6.5 h), varying the depth according to the time and temperature (Table 55.1). About 1 min before sedimentation is complete, lower the tip of a closed Lowry 25 mL pipette slowly into the suspension to the proper depth with a precalibrated Shaw pipette rack. Regulate the filling time of the pipette to about 12 s. Fill the pipette and empty into a tared 90 mL wide-mouth bottle (or 100 mL beaker), and rinse the pipette into the container once.

4 Evaporate the water and dry in an oven at 105°C for at least 24 h. Cool in a desiccator containing phosphorous pentoxide (P_2O_3) or Drierite (calcium sulfate), as a desiccant. Weigh and record the weight.

Determination of Fine Clay (<0.2 μm) (Optional)

1 Pour about 200 mL of suspension from the sedimentation cylinders into 250 mL centrifuge bottles. Shake the suspensions and centrifuge at the appropriate speed for the appropriate time to sediment particles coarser than 0.2 μm to a depth of 5 cm (54 min at 510 *g* at 25°C) on an IEC centrifuge. The formula used is based on Stokes' law:

$$t = \frac{63.0 \times 10^8 n \log R/S}{N^2 D^2 \Delta s} \tag{55.2}$$

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where n is the viscosity in poises (cgs unit of dynamic viscosity equal to 1 dyn s cm^{-2}) at the existing temperature, R the radius of rotation (cm) of the top of sediment in the tube, S the radius of rotation (cm) of the surface of the suspension in the tube, N the revolutions per minute, Δs the difference in specific gravity between the particle in solution and the surrounding liquid (usually use $\Delta s = 1.65$), and t the time in minutes.

2 Withdraw a 25 mL aliquot from a depth of 5 cm. Empty the sample into a tared weighing bottle or beaker, rinse pipette, add the rinsing water to the weighing bottle, dry at 105°C, cool in desiccator, weigh, and record weight.

55.2.4 Calculations

1 A = weight (g) of pipetted fraction (2 or 0.2 μm).

B = weight correction for dispersing agent (g).

Note: To determine the correction factor, add 10 mL of the sodium metaphosphate solution to a 1000 mL cylinder, make to volume, stir thoroughly, withdraw duplicate 25 mL samples, dry, and weigh (about 0.012 g):

$$K = \frac{1000}{\text{volume of pipette (mL)}} \tag{55.3}$$

$$D = \frac{100}{\text{pretreated oven-dried total sample (g)}} \tag{55.4}$$

2 Sand fractions (s):

$$\text{Percentage of sand fraction } (s) = \text{weight (g) of fraction on sieve times } D \tag{55.5}$$

Pipetted fraction (s):

$$\text{Percentage of pipetted fractions } (s) = (A - B)KD \tag{55.6}$$

Silt fraction:

$$\text{Percentage of silt} = 100 - (0{-}2\ \mu\text{m clay} + \text{sand}) \tag{55.7}$$

55.3 HYDROMETER METHOD

A hydrometer can be used to measure the density of a soil suspension after various times of settling and, hence, the particle size distribution. Such measurements can be made on suspensions prepared by any of the pretreatments outlined in Section 55.2.2. In reality, however, the hydrometer is commonly used to estimate particle size distribution without any pretreatment, except dispersion with Calgon®. The hydrometer method outlined here is a simplified version of Day (1965).

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55.3.1 Materials and Reagents

1 Standard hydrometer, ASTM No. 1. 152H, with Bouyoucos scale in g L^{-1}

2 Electric stirrer

3 Plunger

4 End-over-end shaker

5 Cylinders with 1000 mL mark 36 $\pm$ 2 cm from the bottom of the inside

6 Amyl alcohol

7 Calgon® solution (50 g L^{-1}). Contains sodium hexametaphosphate, a dispersing agent

8 Constant-temperature room

55.3.2 Calibration of Hydrometer

1 Add 100 mL of Calgon® solution to the cylinder and make the volume to 1000 mL with distilled water. Mix thoroughly with plunger and let stand until the temperature is constant (between 20°C and 25°C).

2 Lower the hydrometer into the solution carefully, and determine the scale reading R_L at the upper edge of the meniscus surrounding the stem.

55.3.3 Hydrometer Procedure

1 Weigh 40 g of soil (100 g if loamy sand or sandy soil) into a 600 mL beaker, add 100 mL of Calgon® solution, and 300 mL of distilled water, and allow the sample to soak overnight.

2 Weigh another sample of the same soil (10 g) for determination of oven-dried weight. Dry overnight at 105°C, cool, and weigh.

3 Transfer the Calgon®-treated sample to a dispersing cup and mix for 5 min with an electric mixer (milkshake machine), or transfer the suspension to shaker bottles and shake overnight on an end-over-end shaker.

4 Transfer the suspension to a cylinder and add distilled water to bring the volume to 1000 mL.

5 Allow time for the suspensions to equilibrate to room temperature (between 20°C and 25°C).

6 Insert the plunger and move it up and down to mix contents thoroughly. Dislodge sediment with strong upward strokes of the plunger near the bottom and by

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spinning the plunger while the disk is just above the sediment. Finish stirring with two or three slow, smooth strokes. Record the time of completion of stirring. Add a drop of amyl alcohol if the surface of the suspension is covered with foam.

7 Lower the hydrometer carefully into the suspension and take readings after 40 s ($R_{40\,s}$).

8 Remove the hydrometer carefully after the 40 s reading, rinse it, and wipe it dry.

9 Reinsert the hydrometer carefully and take another reading after 7 h ($R_{7\,h}$).

55.3.4 Calculations

$$\text{Sand}\% = 100 - (R_{40\,s} - R_L) \times \frac{100}{\text{oven-dried soil (weight in grams)}} \tag{55.8}$$

$$\text{Clay}\% = (R_{7\,h} - R_L) \times \frac{100}{\text{oven-dried soil (weight in grams)}} \tag{55.9}$$

$$\text{Silt}\% = 100 - (\text{sand}\% + \text{clay}\%) \tag{55.10}$$

55.3.5 Comments

The simplified hydrometer method described in the chapter is not recommended for calcareous or saline soils or soils with greater than 2% organic C. For detailed hydrometer methods please refer to Day (1965).

55.4 SIEVE ANALYSIS (MECHANICAL METHOD)

The grain-size analysis is used in the classification of soils for engineering purposes. The resulting grain-size distribution curves are used as part of the criteria for road engineering, i.e., for road embankment construction or for determining the susceptibility of a soil to frost action.

This grain-size analysis is an attempt to determine the relative proportions of the different grain sizes that make up a given soil mass. This type of analysis has limitations; Day (1965) states that the probability of a particle passing through a sieve in a given time of shaking depends on the nature of the particle, the number of particles of that size, and the properties of the sieve. Gee and Or (2002) suggest that good reproducibility requires careful standardization of the procedure.

55.4.1 Materials and Reagents

1 Sieves and pan (20 cm diameter). Recommended ASTM sieve sizes No. 4 (4.76 mm), No. 10 (2.00 mm), No. 40 (0.42 mm), and No. 200 (0.074 mm)

2 Sieve brush

3 Glass beaker (500 mL)

4 Porcelain evaporation dish (20 cm diameter)

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5 Balance (capacity 1000 g; sensitivity 0.1 g)

6 Mortar and rubber-tipped pestle

7 Drying oven (105°C)

8 Sieve shaker

55.4.2 Procedures

1 Thoroughly clean and weigh each sieve to be used to 0.1 g.

Note: Sieves should always be brushed and cleaned from the bottom side. Particles, which are forced through the sieve from the top, may enlarge the openings and reduce the accuracy and life expectancy of the sieve. Particles, which are stuck in the mesh, may be loosened by tapping the side wall of the sieve against the palm of the hand.

2 Select and weigh a representative sample of approximately 500 g, separate the soil into individual soil particles by crushing with fingers or a rubber-tipped pestle.

The size of sample that is considered to be representative is dependent upon the maximum size fragment present or to be analyzed (Gee and Or 2002). Refer to the list below for representative sample guidelines:

Particles up to 5 mm—500 g

Particles up to 20 mm—5 kg

Particles up to 75 mm—20 kg

Note: For fine-grained soils that dry into hard clods or aggregates, the best and most reproducible method to perform sieve analysis is to take a quantity of oven-dried soil, break the sample as fine as possible, wash on a No. 200 sieve, oven-dry, and sieve the residue through a stack of sieves by shaking horizontally mechanically or by hand for 10 min.

3 The initial washing of the soil should be carefully conducted to avoid damaging the sieve or losing any soil by splashing the material out of the sieve. Wash the soil through the sieve using tap water until the wash water runs clear.

4 Using a wash bottle, carefully back-wash the residue into a large porcelain evaporation dish, decant as much excess water as possible, making sure not to lose any of the sample. Oven-dry the remainder of the soil–water suspension for 16 to 24 h at 105°C.

5 Remove sample from the drying oven, place a watch glass on top of the evaporation dish, and allow the dish and contents to cool to room temperature. Record the weight of the sample (S_w).

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6 Pass the sample through the stack of sieves, using the following sieve sizes: #4, #10, #40, and #200. It is recommended that #20, #60, #100, and #140 sieves also be included in the stack to improve the fit to the semilogarithmic curve.

7 Shake the sample with the sieve shaker for 10 min, weigh each sieve, and record the weight of the sieve plus soil. Subtract the initial weight of the sieve as determined in step 1 and calculate the amount of the total sample retained on each sieve (as a percentage).

8 Sum the total weight of the sieve residues and compare this to the sample weight (S_w) recorded in step 5. If there is greater than 2% discrepancy, the test should be repeated.

9 Calculate the percentage passing each sieve by starting with 100% and subtracting the percent sample retained on each step as a cumulative procedure.

10 Plot a semilogarithmic grain-size distribution curve. If less than 10% of the total sample passes the #200 sieve, the test is finished; if more than 10% passes, then continue with a particle size distribution method.

11 From the grain-size distribution curve calculate the coefficient of uniformity ($Cu = D_{60}/D_{10}$) where D refers to the effective diameter of the soil particles and subscripts (10 and 60) denote the percent which is smaller. An indication of the spread or range of grain size is given by Cu, with a large Cu value indicating that D_{60} and D_{10} sizes differ appreciably.

55.5 NEWER METHODS

Gee and Or (2002) outlined some newer methods of PSA. These include x-ray attenuation, particle counting (Coulter method), and laser light scattering (diffraction). Elias et al. (1999) and Vaz et al. (1999) discussed gamma-ray attenuation while McTainsh et al. (1997) proposed a composite method of PSA involving sieving (>75 μm), Coulter particle counting (2–75 μm), and pipette (<2 μm).

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Chapter 56
Soil Shrinkage

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56.1 INTRODUCTION

Soil shrinkage is the reduction in bulk volume that occurs during drying. It occurs to varying degrees in all soils but most extensively in soils containing appreciable amounts of expanding lattice (smectitic) clays, especially when they are sodic (Parker et al. 1977; Boivin et al. 2004). Also, if a soil shrinks, it can usually swell again, but hysteresis in volume change is common in clays, so shrinkage is not always truly reversible (e.g., Haines 1923; Holmes 1955; Chang and Warkentin 1968). Despite the big differences between shrinkage and swelling processes, however, shrinkage has long been used to indicate a soil's shrink-swell capacity (e.g., Franzmeier and Ross 1968; Ross 1978; De Jong et al. 1992), and to indicate its mechanical workability and its potential for crop performance (Auchinleck 1912). Furthermore, the integrity of engineering structures (Jumikis 1984; Gillot 1986), the potential for pollution of groundwater through cracks (Coles and Trudgill 1985), the regeneration of damaged soil structure (Pillai-McGarry and McGarry 1999), and the availability of soil water under modest overburdens (Groenevelt and Grant 2001; Groenevelt et al. 2001) all depend on the shrink-swell properties of a soil.

Different stages of shrinkage can be identified and the extent of each depends *inter alia* upon whether the soil is poorly or well structured at saturation. For example, when a poorly structured soil begins to lose water from a saturated state, it shrinks. So long as it remains completely saturated during shrinkage, this initial stage of shrinkage is correctly described as ''normal''—the reduction in volume of the soil is exactly equal to the volume of water lost. When interparticle contacts prevent normal shrinkage, cracks form and air enters the soil matrix. Beyond the ''air-entry'' point, shrinkage continues in a manner described as ''residual,'' wherein the reduction in bulk soil-volume gradually declines to values that are less than the volume of water lost. In most soils, shrinkage becomes increasingly restrained and eventually stops beyond the so-called ''shrinkage limit,'' although this ''limit'' may not always occur at a single, clearly defined water content (Groenevelt and Grant 2004). A well-structured soil, however, contains stable macropores, which simply drain and cause little or no loss of bulk volume across a range of water contents known as the ''structural'' shrinkage range. When the structural pores are empty and no further air can enter the soil matrix, it continues to shrink in a proportional fashion such that the reduction in volume is equal to,

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or close to, the volume of water lost—this stage is described as ''basic'' (Mitchell 1992) or ''proportional'' shrinkage (Groenevelt and Grant 2001). Beyond this point, well-structured soils continue to shrink in the residual manner described above.

The methods used to measure shrinkage depend upon the purposes for which the data are collected. For example, data on laboratory soil samples differ significantly from data collected in the field, because large differences exist in the scale at which cracks occur between soil in the field and soil contained in laboratory cores (e.g., Crescimanno and Provenzano 1999), and also because lateral and overburden pressures are present *in situ*, whereas, they often are not in the laboratory (Richards 1986). Furthermore, most field-based measures of shrinkage rely on the existence of a conceptual link between one-dimensional and three-dimensional soil movement, such that changes in elevation or height can be related to changes in volume (Aitchison and Holmes 1953). For most purposes, the assumption of equidimensional shrinkage is probably not unreasonable (Yaalon and Kalmar 1972; Yule and Ritchie 1980a,b), but the link may depend upon the range of soil water contents during which measurements of height are taken (Fox 1964) as well as the sample size relative to the representative elementary volume in the field. For example, measurements of height-change in the field ignore horizontal cracks, which can underestimate shrinkage. In the laboratory, measurements of volume change on small samples exclude cracks altogether, which can overestimate shrinkage. Either way, inherent errors must be acknowledged.

The laboratory methods to measure shrinkage are as numerous as those for particle size analysis. We will consider only two methods here—for further coverage of methods, the reader is referred to McGarry (2002). The field methods, while varying in style, virtually all focus on measuring vertical changes of the bulk soil at different points in the profile relative to a fixed reference point.

56.2 FIELD MEASUREMENT OF SOIL SHRINKAGE

Measuring shrinkage in the field relies heavily upon finding an immovable reference point. Such a point is not always easy to find and you may need to excavate the soil to bedrock or at least well below any zones of expansive soil. Alternatively, it is sometimes possible to gain a clear sighting to a distant reference point that is not affected by expansive soil.

56.2.1 Materials

1. Height-sensing rods of desired length and material. These should be cut with extra length so one end can be threaded coarsely for anchoring in concrete at the depth required and the other end can be cut to protrude from the ground so as to be easily located.

2. Open-casing (metal or PVC) to enclose each rod. The casing is fitted into an auger hole in the soil and the height-sensing rod is driven into wet concrete poured down the hole and allowed to set. The space between the rod and the casing is back-filled with either polystyrene beads (to prevent vapor movement) or heavy-duty grease (to prevent water entry where a water table exists)—either of these fillings also prevent movement of the access tube when the soil around it shrinks and swells.

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3 Annular floating plate (available from most hardware stores) and depth gauge. The annular floating plate is placed over the open-casing and height-sensing rod to sit on the ground and move independently. The depth gauge is placed on the annular floating plate so the vertical movement of the height-sensing rod can be measured with precision.

4 Dumpy level, surveyor's ruler, and datum.

5 Neutron access tube (and neutron moisture meter). Most changes in vertical soil position over time are related to changes in water content, so a neutron access tube (or other moisture-monitoring system that does not disturb the soil profile) should be installed in the vicinity of the height-sensing rods. Assertions can then be made about the extent to which each point in the soil profile contributes to the overall vertical movement of the soil surface. This is particularly important for foundations and footings of engineered structures. An annular floating plate is placed over each tube to allow correction of the depth for each water content reading in case the access tube moves.

56.2.2 Procedures

1 At appropriate intervals in time, the vertical location of each height-sensing rod is checked relative to the annular floating plate using a dial gauge (Figure 56.1).

2 Vertical location of the annular floating plate is then measured relative to the datum using a dumpy level and surveyor's ruler, the operation of which requires two people (one to operate the dumpy level and one to hold the surveyor's ruler on the datum and on the various annular floating plates).

3 Water content of the soil at the depth of each height-sensing rod is measured using a neutron moisture meter. The depths need to be chosen carefully because the average volume of soil represented by each neutron moisture reading is quite large. This requires the height-sensing rods to be placed well above or below horizon boundaries so that average water contents come from uniform layers and can be interpreted.

56.2.3 Comments

The materials for field measurements will vary according to how long the equipment must stay in the ground and what the budget will allow. Salt-resistant and redox-insensitive materials are best for long burial, but appropriate materials vary hugely in price (stainless steel, Teflon, and Perspex are all relatively expensive). Figure 56.1 shows a schematic of the basic equipment involved, but examples showing photos and other schematics of typical equipment used for various purposes can be found in Aitchison and Holmes (1953), Coquet (1998), Braudeau et al. (1999), and Kirby et al. (2003).

Shrinkage generally occurs close to the soil surface where changes in water content are the greatest, but this is not always the case. If water is extracted by plant roots at different depths,

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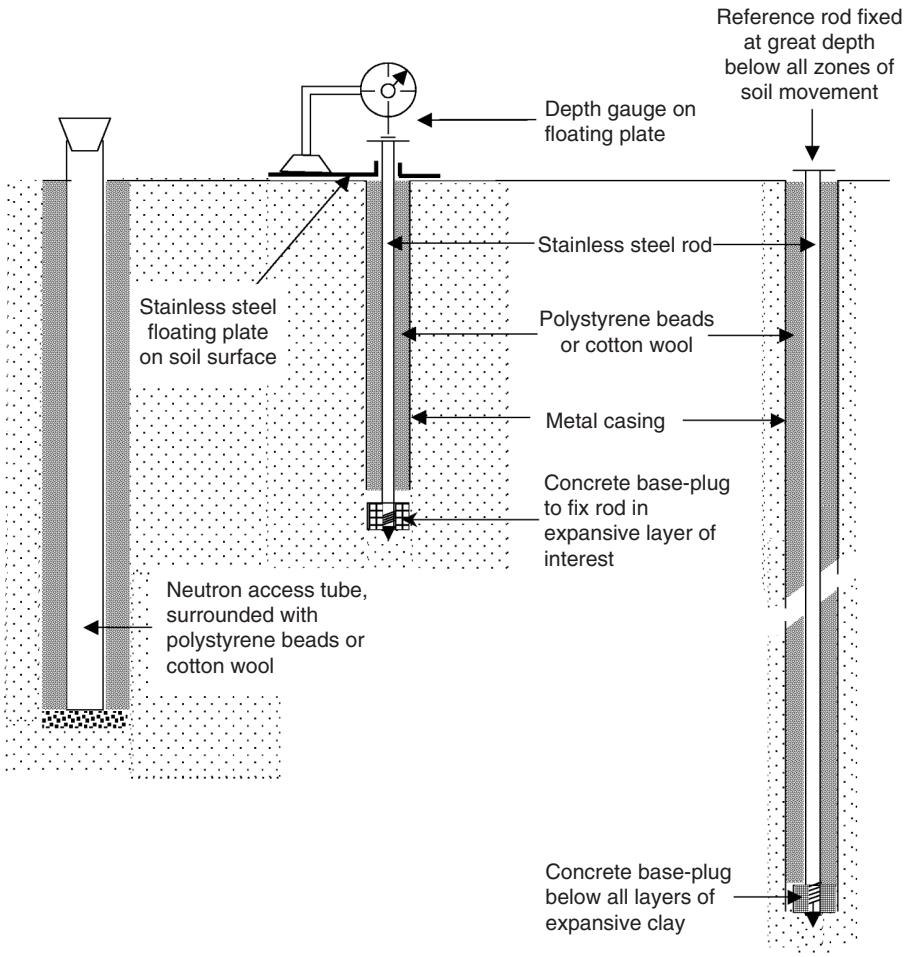
Reference rod fixed at great depth below all zones of soil movement
Depth gauge on floating plate
Stainless steel rod
Stainless steel floating plate on soil surface
Polystyrene beads or cotton wool
Metal casing
Concrete base-plug to fix rod in expansive layer of interest
Neutron access tube, surrounded with polystyrene beads or cotton wool
Concrete base-plug below all layers of expansive clay

FIGURE 56.1. Schematic of instrumentation used to measure vertical soil movement and water content.

it can be difficult to link water content changes with changes in height unless there is knowledge of the bulk densities of the soil at different depths (such information may be available from calibration of the neutron probe for that soil). With this information, adjustments can be made to the water content profile by taking into account the total depth of soil (material depth) in the saturated state (Ringrose-Voase et al. 2000). It should also be noted that the calibration of neutron probes in expansive soils depends upon bulk density, which changes with water content, so is not linear. Greacen et al. (1981) used the following relation to obtain accurate water contents (θ), from neutron probe readings in swelling soils:

$$\theta = \exp\left(\frac{\mathrm{CR} - c}{m}\right) \tag{56.1}$$

where CR is the count ratio and c and m are dimensionless fitting parameters.

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56.3 LABORATORY MEASUREMENT OF SOIL SHRINKAGE

In the laboratory, there are many ways to measure shrinkage depending on equipment available and level of detail required. It is common, however, to measure linear shrinkage because it is simple and repeatable. This involves simply measuring the initial and final lengths of a remolded soil sample as it shrinks from a fixed state of wetness to an oven-dry state. Three-dimensional (volumetric) shrinkage is also measured sometimes, but the procedures involved are more detailed and time-consuming. The choice of method involves balancing the trade-off between rapid and simple procedures, which provide limited information on soil behavior versus more lengthy and complex procedures, which can provide extensive and detailed information from which complex aspects of soil behavior can be extracted (Grant et al. 2002).

56.3.1 Linear Shrinkage

There are several variations of methods to determine linear shrinkage (e.g., McGarry 2002), but a convenient method is to work the soil with water until it reaches a state of consistency known as the Atterberg liquid limit (see Chapter 58). A sample of the remolded soil is then pressed into a special semicylindrical mold of length, L (while tapping to remove air bubbles) and allowed to dry slowly until it reaches a final length at oven-dryness, L_d. The total reduction in length of the sample after drying is expressed as a percentage of the length of the mold, and called ''linear shrinkage'' (LS), as shown in the following equation:

$$\mathrm{LS} = \left(\frac{L - L_d}{L}\right) \times 100 \tag{56.2}$$

Materials

1. Sieve (2 mm), through which the soil should first be passed.
2. Distilled water to mix with soil.
3. Spatula or field knife of appropriate length to work the soil.
4. Large, thick, flat glass plate on which to work the soil.
5. Knowledge of the Atterberg liquid limit (or a device to determine it yourself).
6. Oven, set at 105°C.
7. Semicylindrical mold 2.5 cm internal diameter and 25 cm long (can be shorter), with rectangular ends to contain the wet soil and steady the trough. The mold can be made from any rigid material that will not rust and will not melt at 105°C (e.g., brass or stainless steel). In some cases (e.g., teaching large undergraduate practical classes), such materials may be prohibitively expensive, and it is possible to demonstrate the principles using cheaper materials (e.g., PVC-electrical tubing, split down the middle) so long as the temperature used for oven-drying is not raised above 60°C.

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8 Silicone grease to line the mold to reduce sticking and cracking during drying.

9 Ruler (mm graduations).

Procedures

1 If you already know the soil's water content at the Atterberg liquid limit, determine the air-dry water content of the soil material you plan to use.

2 Take a 200 g sample of the air-dry soil and gradually add sufficient distilled water to make up the difference in water contents between the air-dry state and the liquid limit. (If you do not already know the Atterberg liquid limit, then determine this using current standard procedures—see Chapter 58.)

3 Work the mixture thoroughly with a spatula to obtain the liquid limit paste, then cover the mixture in a sealed container and leave it overnight to equilibrate (check the water content next day to ensure it is still at the liquid limit before proceeding—adjust as necessary with additional water and mix well).

4 Grease the inside of the shrinkage mold of length, L, shown in Figure 56.2a.

5 Fill the mold with the well-mixed soil paste in such a way as to prevent air gaps, and such that the surface is flat and level with the sides of the mold. Clean the edges of the mold with a moist cloth to prevent the soil from sticking to the edges as the soil shrinks (Figure 56.2b).

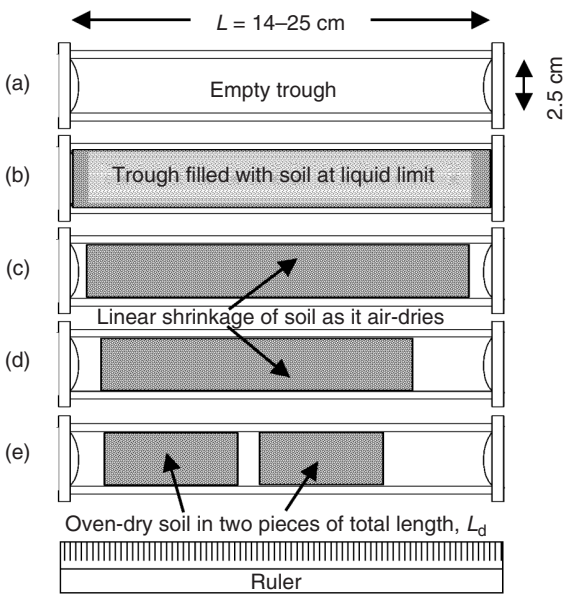

FIGURE 56.2. Trough used to measure linear shrinkage of remolded soil. Letters described in text.

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6 Allow the sample to dry slowly over several days at room temperature (Figure 56.2c and Figure 56.2d).

7 Transfer the mold into an oven at 105°C for 24 h (or for PVC molds—60°C for 1 week), then remove it and allow it to cool.

8 Measure the length of the dry soil in the mold, L_d. If the soil has cracked, assemble the pieces carefully and measure their combined length (Figure 56.2e).

Comments

For highly expansive soils, the final shape of the oven-dry soil in the mold may be cracked and distorted, which makes obtaining a good measure of L_d difficult. One way to avoid excessive cracking and distortion is to prevent the sample drying quickly—perhaps in a sealed container open to the air periodically over weeks instead of days. Put the sample into the oven at 105°C only when you are confident most of the water has evaporated. If you cannot wait that long and you are faced with a broken sample, try to reassemble the bits of soil at the crack-faces and measure their combined length using a piece of string.

Various modifications of the linear shrinkage method have been proposed and are widely used (e.g., Mills et al. 1980; McKenzie et al. 1994). Some methods use finer aggregates (e.g., <425 μm) and different procedures to establish the uppermost water content at which shrinkage begins (e.g., equilibration at a certain matric head following a standardized packing procedure). It is thus important to report the details of any such modifications.

Values of LS correlate well with other measures of shrinkage (described below), and can even be correlated with the tendency of nondispersive, expansive soils to exhibit self-mulching behavior (Grant and Coughlan 2002).

56.3.2 Three-Dimensional Shrinkage

If more detailed knowledge of the shrink-swell characteristics of an expansive soil is required, three-dimensional soil shrinkage is measured by observing the change in bulk mass and volume of a sample as it dries from a wet state. A widely used method for this purpose is the coefficient of linear extensibility (COLE), which was developed by Grossman et al. (1968). The method is not described here, however, because it does not deal with shrinkage from saturation, and furthermore, the method is well described elsewhere (Dasog et al. 1988; Warkentin 1993; Soil Survey Staff 1999; McGarry 2002).

This section focuses on the detailed measurement of three-dimensional shrinkage from total saturation to oven-dryness. First, however, a few things need to be appreciated before embarking on this measurement, because to do it well can take many weeks.

Measuring the mass of a soil sample is a trivial matter, at least in concept if not in practice (e.g., very wet samples can be difficult to weigh because they deform easily during handling, but if the sample is stable its mass can easily be recorded). I have found that samples are best contained in Teflon rings to avoid deformation during handling and to prevent samples from sticking to the edges of the ring as they shrink.

The primary challenge in monitoring three-dimensional soil shrinkage is to obtain accurate measures of the bulk volume of a sample. Measuring the linear dimensions of a cube or

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cylinder or other regular shape of soil is relatively simple (Schafer and Singer 1976)—good instruments are available for this purpose, such as Vernier callipers, traveling microscopes, and cathetometers to name a few. The volume of a regularly shaped sample can be calculated with reasonable precision and accuracy from the linear dimensions, at least during the early stages of shrinkage. For irregularly shaped samples, however, the error in estimating volume by estimating the linear dimensions increases as the cube of the error in the measured lengths. Most studies simply ignore this problem and do not report linear measurement errors. Remolded samples that start with regular shapes (disks, cubes, etc.) often deform during drying and lose their regular shape—greater errors occur in determining the volume of such samples, particularly if they are small.

Archimedes' principle tells us that when a solid object is immersed in an incompressible fluid (e.g., water), it will displace a mass of the fluid equivalent to the volume of the object. This is because an object experiences buoyancy when immersed, so its mass appears to decline. The apparent weight loss of the solid immersed in the fluid is directly proportional to the volume of the fluid displaced. The sample is usually coated with a thin layer of Saran resin, which prevents liquid water being taken up but allows water vapor to diffuse out during shrinkage (Warkentin 1993). If applied thinly, the correction required for the volume of the Saran coating can be as small as 4.6% (McGarry 2002). The volume of the sample, V_{sample}, can be calculated using the following equation:

$$V_{\text{sample}} = V_{\text{water}} = \frac{M_{\text{sample(air)}} - M_{\text{sample(water)}}}{\rho_{\text{water}}} = \frac{M_{\text{water}}}{\rho_{\text{water}}} \tag{56.3}$$

where V_{water} is the volume of water displaced by soil sample, M_{water} the mass of water displaced by soil sample, $M_{\text{sample(air)}}$ the mass of soil sample measured in air, $M_{\text{sample(water)}}$ the mass of soil sample measured in water, and ρ_{water} the density of water at ambient temperature.

A satisfactory alternative to coating the sample with Saran is to case the sample in a thin rubber membrane such as a balloon or prophylactic (Tariq and Durnford 1993). The apparatus shown in Figure 56.3a through Figure 56.3c, enables the sample to dry slowly

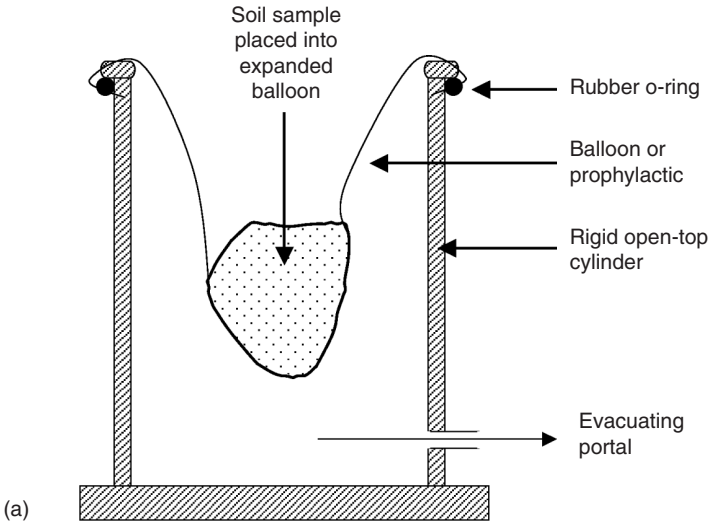

FIGURE 56.3. (a) Suction cylinder used to fit a thin rubber membrane around a soil sample.

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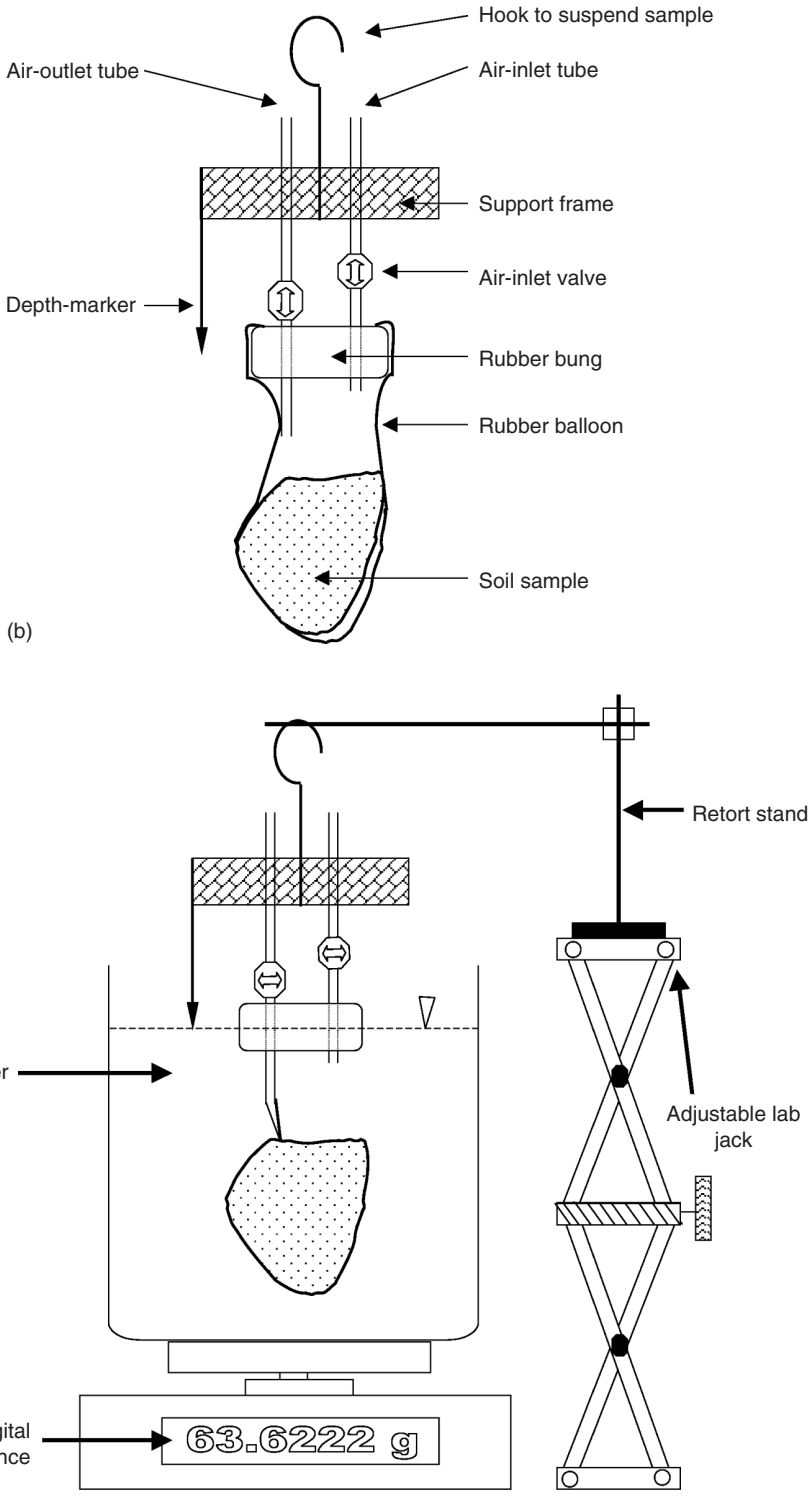

FIGURE 56.3. (continued) (b) Apparatus for supporting the sample and drawing air across it to dry it in its membrane. (c) Assembly to determine the volume of the soil sample by displacement as it shrinks.

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without handling it or the membrane at any time during shrinkage, and allows easy removal for obtaining the oven-dry weight.

Materials

1. Rigid (e.g., brass) open cylinder (called a ''suction cylinder'' used by engineers in association with triaxial apparatus) with rubber o-ring at top and an evacuating portal near the base (Figure 56.3a). This needs to be large enough to hold the soil sample yet small enough so that a thin rubber membrane (e.g., balloon or prophylactic) can be stretched over the rim and held firmly by a rubber o-ring.
2. Large, thin-walled balloon or prophylactic. The mass and volume of the rubber membrane, when stretched over the soil sample, are very small and may be negligible.
3. Materials to make up a sample-support structure with rigid air-inlet and -outlet tubes as shown in Figure 56.3b.
4. Laboratory retort stand and adjustable laboratory jack.
5. Digital top-loading balance of 1 kg capacity.
6. 1000 mL beaker or other container.
7. Source of dry air, with pressurized adjustable flow rate.
8. Soil clod, of any volume in the range 30–500 cm^3 (larger is better).
9. Water at same temperature as soil sample.
10. Other materials shown in Figure 56.3c.

Procedures

1. Assemble the materials shown in Figure 56.3a through Figure 56.3c.
2. Use the rigid open cylinder and apply a small suction to expand the balloon against the sides of the cylinder while you place the soil sample in it.
3. Release the suction to allow the balloon to fit firmly around the sample, ensuring no air gets entrapped.
4. Remove the sample from the suction cylinder and wrap the neck of the balloon over the rubber bung shown in Figure 56.3b such that the air-inlet and -outlet tubes are not blocked.

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5 Close off the air-inlet valve and open the air-outlet valve. Apply suction to exclude all air and to make the balloon fit tightly against the sample.

6 Weigh the sample to obtain the weight in air, $M_{sample(air)}$.

7 Hang the assembled sample onto the retort stand and lower it into a beaker of water sitting (tared) on a top-loading electronic digital balance. Record the weight, $M_{sample(water)}$, when you have lowered the sample to a consistent depth indicated by the depth-marker in Figure 56.3c.

8 To dry the sample, lift it from the beaker, open both air valves, and apply a steady, small positive pressure down the air-inlet tube; the air passes over the soil sample and escapes through the air-outlet tube.

9 Control the rate of shrinkage by adjusting the flow of air over the sample.

10 As shrinkage occurs, measure the volume at periodic intervals by repeating procedures 5 to 7.

11 When shrinkage ceases, remove the sample from the membrane, dry it at 105°C and measure the oven-dry mass and volume.

12 Calculate the sample volume for each stage using Equation 56.3. Use the data to calculate the void ratio, e (volume of pores per unit volume of solids) and the moisture ratio, ϑ (volume of water per unit volume of solids) using the following equations:

$$e = \frac{V_{pores}}{V_{solids}} \tag{56.4}$$

where

$$V_{pores} = V_{sample} - V_{solids}$$
$$V_{solids} = \frac{M_{OD\ solids}}{\rho_{solids}}$$

where $M_{OD\ solids}$ is the oven-dry weight of sample.

$$\vartheta = \frac{V_{water}}{V_{solids}} \tag{56.5}$$

where

$$V_{water} = \frac{M_{water}}{\rho_{water}}$$

where M_{water} is the mass of water remaining in soil sample at each stage of shrinkage and ρ_{water} is the density of water at ambient temperature, often assumed to be 1 g cm^{-3}.

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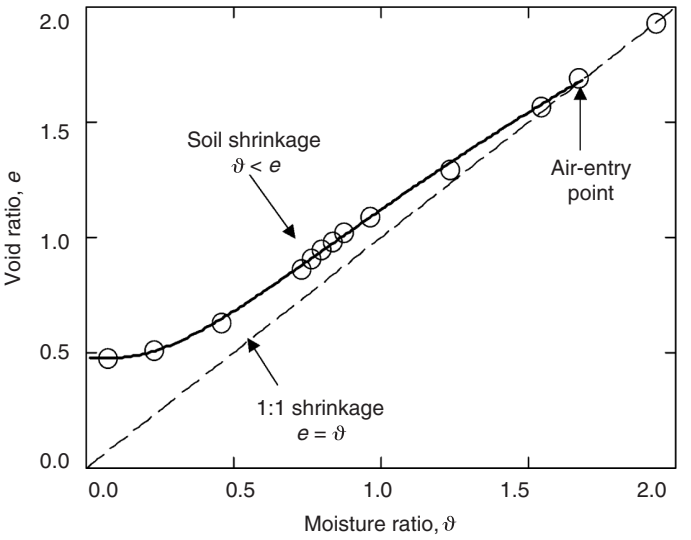

FIGURE 56.4. Typical shrinkage curve showing the air-entry point and the line of shrinkage taken by the soil relative to the 1:1 line.

13 Plot the void ratio as a function of the moisture ratio for each stage of shrinkage. This will produce a ''shrinkage curve,'' which can be superimposed on a 1:1 line to identify the point of air entry as shown in Figure 56.4.

Comments

1 To obtain both the mass and the volume of an irregularly shaped soil sample by direct measurement and without disturbing or handling the sample is a challenging prospect. The method outlined here presents the fewest technical problems in this regard but it is still imperfect. A range of different rubber membranes needs to be kept on hand because sample size and shape vary. The thickness and integrity of the rubber membrane must be sufficiently thin and flexible to hug all external macropores, yet thick enough not to tear. Prophylactics from the supermarket seem to offer as much scope as any rubber material available, but these vary from place to place and with time so it is a tedious exercise to find a suitable range. The suction cylinder helps to obtain a good fit with the soil sample, but practice is required to avoid small gaps.

2 Samples raised from the soil profile and brought into the laboratory to measure shrinkage (e.g., Reeve and Hall 1978; Prebble 1991) behave differently than they would under their natural overburden (Groenevelt et al. 2001). A load equivalent to the natural overburden needs to be applied during measurement of shrinkage in the laboratory; otherwise, the matric head needs to be corrected. It is theoretically possible to correct the matric head using the unloaded shrinkage line (e.g., Groenevelt and Bolt 1972; Stroosnijder 1976; Groenevelt and Kay 1981) but almost no data exist to corroborate this and all of it comes from remolded pastes (e.g., Talsma 1977), not undisturbed soils. To avoid having to correct the matric head, an overburden could be applied to samples using the technique outlined here with only minor modification. It should be possible to encase the entire

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apparatus shown in Figure 56.3b and immerse it underwater to a depth equivalent to the overburden. The air-inlet and -outlet tubes would require consideration to keep them open during air flow, or it might also be possible to eliminate the need for one of the tubes by coating the sample with a porous membrane and immerse the sample in a fluid for which the osmotic head can be adjusted (e.g., polyethylene glycol [PEG]). This would achieve a known osmo-matric head (cf. Groenevelt et al. 2004), while at the same time allowing the natural overburden to be applied. A clever student could work out the technical details.

3 The shrinkage curve can be analyzed in a number of ways to obtain shrinkage limits, plastic limits, and other important structural characteristics of expansive soils (cf. Groenevelt and Grant 2002; Peng et al. 2005; Cornelis et al. 2006).

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Chapter 57
Soil Density and Porosity

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57.1 INTRODUCTION

Soil is composed of solid particles (minerals and organic matter) of different sizes, usually bound together into aggregates by organic matter, mineral oxides, and charged clay particles. The gaps between the particles are linked together in a complex network of pores of various sizes. The total pore space of a particular soil consists of pores existing both between adjacent soil particles and between soil aggregates. Through this pore space the soil exchanges water and air with the environment. The movement of air and water also allows for heat and nutrients to flow. The number and size of pores vary considerably among soils exhibiting different organic matter content, texture, and structure.

Soil bulk density ($D_b = m_s/V_t$) is the ratio of the mass of oven-dried solids (m_s) to the bulk volume (V_t) of the soil, which includes the volume of the solids and the pore space between the soil particles (Blake and Hartge 1986a). D_b is a widely used physical property; it is a

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measure of soil compaction status and is needed for converting water percentage by weight to content by volume, for calculating porosity when particle density is known, and for estimating the weight of a volume of soil too large to weigh conveniently.

The particle density ($D_p = m_s/V_s$) refers to the mass (m_s) of a unit volume of solid soil particles (V_s). No account is taken for the pore space between the particles (Blake and Hartge 1986b). Particle density is used in most mathematical expressions where the volume or weight of a soil sample is being considered. Thus, interrelationships among porosity, bulk density, air space, and rates of sedimentation of particles in fluids depend on particle density.

Porosity (S_t) is the percentage of the soil volume occupied by pore spaces. The types of pores present in a soil are as important as the amount of pore space. Pore spaces are filled with air (F_a, air-filled porosity) or water (WFPS or relative saturation). Pore space is divided into different categories by pore diameter, especially the large soil pores that are associated with the transfer and movement of both water and air (Carter and Ball 1993).

Agricultural activities, which could involve tillage or the wheels of heavy machinery compacting soils, can have a great effect on bulk density and porosity (Gysi et al. 2000; Cameira et al. 2003; Osunbitan et al. 2005). Any management practice that increases organic matter will increase the granular structure of the soil, increase the pore space, and decrease the bulk density (Shaver et al. 2003). Soil density and porosity are the two most important parameters in assessing anthropogenic change in soil.

In the last decade, progress has been made in the determination of soil bulk density and porosity. One of the newer methods is computerized tomography (CT) based on image reconstruction techniques that use x-rays and γ-rays as a source of radiation (Pedrotti et al. 2005). This method has been used to measure soil physical properties, such as bulk density, water content (Crestana and Vaz 1998; Pedrotti et al. 2005), porosity, soil fracture size, geometry and topology of macropore networks (Perret et al. 1999), and micropore size (Macedo et al. 1998). Time domain reflectometry (TDR) probe methods are also being used to simultaneously measure water content, air-filled porosity, and bulk density (Ochsner et al. 2001). However, the focus of this chapter is on presenting common and easy to use methods that require simple and inexpensive equipment.

57.2 SOIL BULK DENSITY

The D_b is a dynamic soil property. D_b differs from D_p in that a measure of bulk density includes all pore space. Unlike D_p, which expresses the density of the solid soil constituents only, D_b is strongly influenced by the quantity and size of the pore spaces as well as the composition of the solid soil materials. As a result, loose, porous soils will have lower bulk densities than more compact soils. Sandy soils, which are relatively low in total pore space and are often low in organic matter content, typically have higher D_b values.

Except for recently tilled soil, D_b is considered to exhibit relatively low spatial variability (Warrick and Neilson 1980). Generally, coefficients of variability for measurements of D_b for a given profile horizon of a soil series do not exceed about 10% of the mean (Grossman and Reinsch 2002). Thus, about four samples per depth and per field of treatment should be sufficient to estimate the mean density to within 10% of the true values, 95% of the time, for a uniform soil type.

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Soil D_b can be determined by either sampling or *in situ* methods. For sampling methods, which involve disturbing the soil and removing some of it, the most common is the core method while the clod and excavation approaches are also used. Alternatively, the nuclear radiation method will not disturb the soil being sampled. These four methods are described in this section. Different methods have different personnel and capital costs.

57.2.1 Core Methods

Materials and Supplies

1. Double-cylinder core sampler, either hand operated or hydraulically driven
2. Clean, dry, and uniform cylinder with a known internal diameter d (cm) and height h (cm). The volume V (cm^3) is

$$V = \frac{1}{4}\pi d^2 h \tag{57.1}$$

3. Sharp and rigid knife or spatula
4. Balance sensitive to 0.01 g
5. Drying oven capable of 105°C, preferably equipped with a circulating fan
6. Plastic bags and corrosion-resistant weighing tins large enough to hold the soil sample and the cylindrical core
7. Disks to protect the ends of cores
8. Masking tape

Procedures

1. Label and weigh cylindrical core sampler; record weight as W_1 (g).
2. Label tin bottom and top, weigh together, and record weight as W_2 (g).
3. Prepare a smooth ''undisturbed'' vertical or horizontal surface at the sampling depth.
4. Drive or press core sampler into the soil sufficiently to fill the inner core without inducing compression. Do not rock the sampler. In frictional or dense soils, careful excavation to minimize soil–metal adhesion may help in obtaining a representative core. An application of mineral oil to the core sampler may also be beneficial. Use of oil may affect wetting and drying within the core if it is to be used for water desorption characterization.
5. After careful removal of the undisturbed soil core, examine for signs of shattering or compression. Trim ends of acceptable soil cores flush with the end of the cylinder. Discard and resample if substantial root biomass or large coarse fragments protrude. Remove organic matter thatch at surface before sampling.

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6 For cores that completely fill the cylinders, and if only density is to be determined, push the content of the cylinder out into a preweighed tin, which is then closed and weighed (W_3, g).

7 Place samples in an oven set to 105°C. Drying time varies with core size and oven type. Cores of about 350 cm^3 usually require about 72 h of drying in ovens equipped with circulating fans. Smaller cores require less time. After drying and cooling in a desiccator, record the weight of the dry soil plus tin bottom and top as W_5 (g).

Calculations

$$D_b = \frac{W_5 - W_2}{V} \tag{57.2}$$

and volumetric water content θ is

$$\theta = \frac{W_3 - W_5}{W_5 - W_2} \times \frac{D_b}{D_w} \tag{57.3}$$

The wet density (D_{bw}), used in soil mechanics and also for making comparisons between samples of soil that exhibit volume changes on drying, is

$$D_{bw} = D_b + \theta(\text{g cm}^{-3}) \quad \text{or} \quad \frac{W_3 - W_2}{V} \tag{57.4}$$

Density of Partially Filled Cores

1 Tare a graduated cylinder of volume V_{g1} and then fill with glass beads. The weight of the beads is recorded as W_{g1}.

2 Obtain the weight (W_4) of the partially filled soil core, place a disk under one end, and put it on a tray.

3 Pour glass beads onto the soil and level to the top of the cylinder with a spatula.

4 Place a disk over the top of the cylinder, invert the core, and fill the other end with beads. Transfer the core to a preweighed tin (W_2) and dry at 105°C.

5 Return excess glass beads from the tray to the cylinder and record their volume and weight as V_{g2} and W_{g2}, respectively.

6 After drying the sample, cool in a desiccator and weigh (W_5).

7 Calculate soil volume V_s as

$$V_s = V - \frac{W_{g1} - W_{g2}}{C} \tag{57.5}$$

where C is the packing density of the glass beads, which should be verified for each analysis. Beads having a nominal diameter of 260 μm pack to a density

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of about 1.50 g cm^{-3}. Alternatively, the volume of beads can be calculated as $V_{g1} - V_{g2}$; so that $V_s = V - (V_{g1} - V_{g2})$.

8 Calculate D_b as

$$D_b = \frac{W_5 - (W_{g1} - W_{g2}) - W_2 - W_1}{V_s} \tag{57.6}$$

and θ as

$$\theta = \frac{W_4 - W_1}{V_s \cdot D_w} \tag{57.7}$$

Correction for Coarse Fragments

For certain applications, the D_b of the fine fraction, defined as those particles less than 2 mm in diameter, is of interest. This density is obtained by sieving the soil through a 2 mm sieve, then oven-drying the soil that passed through. The material retained on the sieve is washed, dried, and weighed (recorded as W_6). The volume of this fraction, V_c, can be determined by measuring the displacement of water in a graduated cylinder when the fragments are added. D_b is then

$$D_b = \frac{W_5 - W_2 - W_1 - W_6}{V - V_c} \tag{57.8}$$

where W_6 is weight and V_c is the volume of oven-dry soil >2 mm in size, and W_1, W_2, W_5, and V are defined in the Section Procedures, pp. 745–746 in steps 1 to 7.

Comments

1 Core compaction accounts for the largest error inherent in this method. To avoid compression or disturbance in heavy, compact, or plastic soils, core diameters should be >7.5 cm with the height of the core not exceeding the diameter. This minimizes disturbance at the core edge, especially when the core samples will be used for further measurement such as pore-size distribution or hydraulic conductivity (Blake and Hartge 1986a). If bulk density is the only parameter being measured, the most common core diameters range from 5 to 7.5 cm.

2 In addition to double cylinders, other tools have been used to obtain soil samples for density determination. For example, McCauley peat augers, which remove a half cylinder, are often used in peat soils.

3 The core method is relatively cheap, requiring only a coring device, a balance, and an oven. Personnel costs are high since sample collection is time-consuming. Sampling deeper into the soil profile often requires two people.

4 Measurements of D_b using destructive sampling methods are influenced by soil water content, particularly, in soil that exhibits high shrinkage and expansion when water content changes. Thus, water content measurement should always accompany D_b determination (Blake and Hartge 1986a).

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57.2.2 Clod Method

Blake and Hartge (1986a) and Grossman and Reinsch (2002) have presented details of the clod method. The procedure involves the application of Archimedes' principle to determine clod volume. Bulk density is determined by weighing the clod, coating or saturating it with a water-repellent substance (e.g., paraffin wax or saran resin), and then weighing the coated or saturated clod both in air and while immersed in a liquid of known density and temperature (Campbell and Henshall 2001). A portion of the clod is removed and weighed before and after oven-drying at 105°C to calculate D_b as corrected for water content.

The clod method is time-consuming. Only quite stable (and cohesive) aggregates should be used, and as Blake and Hartge (1986a) pointed out, even then it is not very precise. The whole aggregate, rather than just a subsample, is oven dried at 105°C. The clod method assumes that about 10% of saran initially present is lost on drying. This assumption does not strongly affect results. Shrinkage over specific water content ranges of interest can be determined by equilibrating clods on pressure plate or tension tanks before coating with saran.

Small clods are not appropriate for this method, as the macropore space between clods and the larger fragments of coarser soils are not included in the measurement. Choosing representative clods from disturbed soil layers is difficult because of packing by equipment (Blake and Hartge 1986a).

57.2.3 Excavation Methods

Excavation, developed by soil engineers for bituminous and gravely material, is utilized in tillage and forestry research where loose surface soil and stones prevent the use of core and clod methods (Blake and Hartge 1986a). Bulk density is determined by excavating a quantity of soil, drying and weighing it, and determining the volume of the excavation by filling with an inert substance of known packing density (Blake and Hartge 1986a; Campbell and Henshall 2001; Grossman and Reinsch 2002).

57.2.4 Nuclear Radiation Methods

Nuclear radiation methods provide a rapid means of measuring wet bulk density with suitable calibration. Gardner (1986), Grossman and Reinsch (2002), and Campbell and Henshall (2001) have discussed the principles of γ-ray attenuation to determine soil D_b as well as the sources of error associated with its measurement. Briefly, D_b is determined by measuring the scattering or transmission of γ-rays between a source and detector. This scattering or transmission varies with soil properties, including density. The scattering technique employs a single source and a detector located on either a surface gauge or single probe. Transmission (attenuation) techniques use two rods with the gamma source located on one rod and the Geiger detector on the other. Probes are inserted into access tubes or into predrilled holes in the soil to measure scattered or transmitted radiation. Soil water is measured concurrently to convert D_{bw} to D_b. The dual source (combined gamma–neutron probe) simultaneously measures density and water with corrections required for D_b.

Commercially available gauged methods are increasingly used for determining D_{bw} and water content. For example, the Campbell Pacific Nuclear Dual Probe Strata Gauge MC-S-24

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is equipped with two radioactive sources: a Cs_{137} (370 MB_q) gamma-emitter for determination of D_{bw} and an Am_{241}–Be (1900 MB_q) source, emitting fast neutrons, for water content determination. D_{bw} and water content are automatically calculated for direct read-out on the gauge display. The D_b is calculated by subtracting the volumetric water content from D_{bw}.

Manufacturers supply detailed instructions on the safe use of these instruments. Please follow the manufacturer's instructions for determining standard and measured counts. The equipment should be checked for radiation leaks on a regular basis.

Nuclear radiation methods are nondestructive, and are particularly useful when taking intact soil cores is difficult. They are efficient, enabling one person to take many readings in a short time. But this technique has a high capital cost. There is also a need to consider the registration cost for radiation safety monitoring, and requirements for special transport, training for nuclear and safety protocols, and finally, nuclear source disposal.

57.3 SOIL PARTICLE DENSITY

The D_p of a soil represents the composite average of the density of all the particles that comprise the soil. The density of individual soil particles is dependent on their mineralogy and composition. The density of minerals commonly found in soils varies from 2.6 to 2.75 g cm^{-3}. Quartz, feldspars, and colloidal silicates predominate in mineral soils and their densities fall within this range. However, if unusually higher amounts of ''heavy'' minerals such as magnetite, zircon, tourmaline, and hornblende are present in a soil, the particle density of the soil may be greater than 2.75 g cm^{-3}. Particle density can vary widely between soils, even within a soil series. Particle density may also vary with position on a slope, due to the variability in clay mineralogy (Ball et al. 2000) associated with tillage or with variation in type and content of heavy minerals. Ball et al. (2000) recommend that particle density be measured whenever it is to be used in calculating porosities (Section 57.4.1). The particle density of organic matter is far less than mineral particles, typically ranging from 1.3 to 1.5 g cm^{-3}. Thus, relatively small amounts of organic matter can have a considerable effect on a soil's composite particle density. Surface soils, because of their higher organic matter content, usually possess lower particle densities than subsoil.

A soil D_p of 2.65 g cm^{-3} is commonly assumed. This value corresponds to the D_p for quartz. For a soil consisting of three constituents x_1, x_2, and x_3 (fraction expressed by weight) with particle densities of D_{p1}, D_{p2}, and D_{p3}, soil D_p can be calculated as follows (Culley 1993):

$$\frac{1}{D_p} = \frac{x_1}{D_{p1}} + \frac{x_2}{D_{p2}} + \frac{x_3}{D_{p3}} \tag{57.9}$$

The D_p of a soil sample is calculated from two measured quantities, the weight and volume of particles. The weight is determined by weighing and volume by calculation from the weight and density of water (or other fluid) displaced by the sample.

57.3.1 Materials and Supplies

1 Pycnometer (or 100 mL volumetric flask)

2 Distilled water

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3 Thermometer

4 Air-dried soil sieved through a 2 mm sieve

5 Balance sensitive to 0.001 g

6 Drying oven capable of 105°C

57.3.2 Procedure

1 Dry duplicate soil samples in a 105°C oven beforehand to determine the gravimetric water content (θ_w) of air-dried soil.

2 Degas distilled water by gently boiling for several minutes and cooling to room temperature. Record the temperature of this water and corresponding density of water (D_w) at this temperature.

3 Fill a pycnometer with the degassed water. Insert the stopper in the pycnometer. Ensure the capillary bore in the stopper is filled. Wipe the pycnometer bottle dry and weigh (W_w).

4 Pour out about half of the water from the pycnometer. Replace the stopper, dry the outside of the bottle, and weigh it.

5 Add approximately 10 g of air-dried soil and again weigh the pycnometer and stopper. (*Note*: the difference in weights obtained in (5) and (4) is the weight of the air-dried soil, W_a). The weight of the oven-dried soil W_s is

$$W_s = \frac{W_a}{1 + \theta_w} \tag{57.10}$$

6 Refill the pycnometer with water. Replace the stopper and again make sure that the capillary bore is filled. Weigh the pycnometer, water, and soil (W_{sw}).

57.3.3 Calculation

$$D_p = \frac{d_w W_s}{W_s - (W_{sw} - W_w)} \tag{57.11}$$

57.3.4 Comments

Note: if a 100 mL volumetric flask is used, add 50 g of air-dried soil and follow all procedures as for a pycnometer.

57.4 SOIL POROSITY

Knowing the number, size, configuration, and distribution of soil pores is useful for assessing the physical condition and structure of the soil (Carter and Ball 1993), but classification of pore sizes lacks standardization. In many cases, pore-size distribution is considered the best indicator

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of the soil physical condition. Associated porosity factors, such as macropore volume, pore continuity, and air-filled pore space are also important guides to characterize soil structure.

The texture and arrangement of solid soil particles determines soil porosity. Porosity of sandy surface soils may range from 35% to 50%, whereas finer textured soil typically ranges from 40% to 60%. Compact subsoils may have as little as 25%–30% total pore space. Bulk density values generally reflect soil porosity. In general, the higher the D_b, the lower the porosity. Sandy soils are dominated by large pores. Thus, movement of air and water through sandy soils is relatively unrestricted and rapid in spite of the small amount of total pore space. Fine-textured soils are dominated by micropores, and although total porosity is greater in fine-textured soils compared to soils of coarser texture, the smaller size of the micropores restricts movement of air and water.

Pore-size distribution has proven useful for predicting water infiltration rates, water availability to plants, water storage capacity, aeration status (Cary and Hayden 1973), and classification of soil structure (Thomasson 1978; McKeague et al. 1986). Macropores mainly facilitate the flow of water when soil is saturated. Pore-size distribution is useful for predicting hydraulic conductivity (Suleiman and Ritchie 2001). There is a strong relationship between saturated hydraulic conductivity and macroporosity (effective porosity) (Aimrun et al. 2004).

Pore-size distribution in soil provides descriptive information about the soil pore system, rather than absolute measurements. The techniques are limited by the basic assumptions of the capillary model, which represents soil pores as parallel tubes of varying radii (Ball 1981a), and by nonstandard terminologies for classifying the various pore sizes (Danielson and Sutherland 1986). Pore sizes are thus expressed as equivalent pore diameters (EPD).

Recent developments in computers and image-analysis software make possible the analysis of soil macroporosity with technology such as ultrahigh resolution x-ray tomography (Beaudet-Vidal et al. 1998; Gantzer and Anderson 2002). Micromorphological methods can be used to characterize the actual morphology of soil pores, and are especially useful for studying the shape and continuity of pores above 100 μm in diameter. Morphological techniques are also used to describe large pores and cracks (greater than 2 mm diameter or width) such as wormholes and pores between large structural units.

This section describes the most common and easy to use methods for measuring total porosity, its air- and water-filled fractions, and pore-size distribution from measurements of water desorption using soil cores. The latter is suitable for determination of equivalent pore sizes below 150 μm (Bouma 1991). Indices of pore continuity from measurements of gas diffusion or air permeability are also discussed.

57.4.1 Total Porosity

The total porosity S_t may be calculated from the particle density and bulk density as

$$S_t = 1 - \frac{D_b}{D_p} \tag{57.12}$$

The apparatus and experimental procedures used for measuring D_p and D_b are described in Sections 57.2 and 57.3. Results for S_t can be expressed as a volume fraction or as a percentage. In this chapter, percentages will be used to represent porosity indices.

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57.4.2 Macroporosity or Effective Porosity

Macropores are the primary pathway to conduct the flow of water when the soil is saturated. Effective porosity ($Ø_e$) is a measure of this property. It is approximately equal to the difference between total porosity and volumetric water content at 33 kPa of suction or volumetric water content at field capacity (Yu et al. 1993) where heavy clayey soils can be considered at 66 kPa of suction. Deeks et al. (2004) define macropores as those having nominal diameter >50 μm whereas micropores are <50 μm, equivalent to 6 kPa of suction.

The $Ø_e$ could be used to estimate the saturated hydraulic conductivity of soil (Aimrun et al. 2004) since measurement of K_s is time-consuming, labor intensive, and expensive. The $Ø_e$ is a useful index to gauge soil response to different management and tillage systems (Carter 1988). Generally, $Ø_e$ should exceed 10% of the soil volume to maintain optimal soil aeration. However, this can be modified by the degree of pore continuity.

57.4.3 Air-Filled Porosity

Air-filled porosity is a measure of the fraction of the soil bulk volume occupied by air. Soil matric potential information should be given when reporting air-filled porosity. Assuming that the total pore space is filled with water at saturation, the air-filled pore space at −6 kPa matric potential would equal the volume of macropores based on the definition given by Deeks et al. (2004).

Air-filled porosity (F_a), as a percent of total soil volume, can be calculated from the tension table procedure for any specific matric potential using the following equation:

$$F_a = \frac{W_s - W_p}{V_t} \times 100 \tag{57.13}$$

where W_s is the saturated core weight, W_p is the core weight at a specific potential, and V_t is the core volume. The difference method based on bulk density and soil water content can also be used for determining the F_a (volume basis) of a core at any moisture content by calculating total porosity (S_t) using Equation 57.12 and the following equation:

$$F_a = S_t - \theta_w D_b / D_w \tag{57.14}$$

where θ_w is gravimetric moisture content. Ball and Hunter (1988) describe some of the limitations and errors involved in the determination of air-filled porosity.

57.4.4 Water-Filled Pore Space or Relative Saturation

Water-filled pore space (WFPS), sometimes called ''relative saturation,'' expresses the volume of water in the soil relative to the total volume of pores. Thus, it ranges from 0% in a dry soil to 100% under saturated conditions. A relative saturation over 65%–70% can indicate that the soil may become anaerobic (Linn and Doran 1984) and has proved a useful index for porosity studies in wet soils (Carter 1988). Relative saturation is calculated in soil cores as follows:

$$\text{WFPS} = \frac{W_w \Big/ \left(V_t - \dfrac{W_s}{D_p}\right)}{D_w} \tag{57.15}$$

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where W_w is the weight of water (g), W_s is the dry weight of soil (g), and V_t, D_p, and D_w are defined as in Section 57.1.

The WFPS index can also be calculated from F_a and S_t (i.e., WFPS $= (S_t - F_a)/S_t$).

57.4.5 Pore-Size Distribution

Pore-Size Classification

Attempts have been made to classify pores in regard to function rather than size alone. Various terminologies for EPD are used to describe the functional properties of pore-size groups as follows: fissures (>500 μm), transmission pores (500–50 μm), storage pores (50–0.5 μm), and residual pores (<0.5 μm). Emphasis has also been placed on large pores (termed ''macropores''), above 50 μm in diameter, which are associated with saturated hydraulic conductivity (Germann and Beven 1981). Generally, the range of pore-size of interest will depend on the purpose of the measurement (Ball and Hunter 1988). For example, soil-survey purposes may require pore-size ranges associated with field capacity (about 50 μm) and the lower limit of readily available water for plants (about 3–1.5 μm). Thomasson (1978) used the soil volume of pores greater than 60 μm diameter (termed ''air capacity'') and the volume occupied by pores of between 60 and 0.2 μm (termed ''available water'') as a classification of soil structural condition. In contrast, soil structure or tillage studies require information about macroporosity (>50 μm). Studies on infiltration and preferential flow of water require information on pores >1 mm EPD in addition to lower pore sizes (Luxmoore et al. 1990).

Pore-Size Distribution

Precise evaluation of the size, configuration, and distribution of the soil pores is essentially impossible due to their complicated nature. However, by making certain assumptions, the size distribution of the larger pores can be measured with at least useful accuracy. Starting with saturated soil samples, drain the soil cores stepwise and measure the volume of water removed between consecutive steps. The volume of water removed can be equated to the soil pore-volume drained. Then, if the size-range of pores drained during each step can be calculated, the pore-size distribution can be determined. In theory, the largest pores should drain first, followed by successively smaller and smaller pores. Actually, the drainage of the linked pore system will be determined by the diameter of the ''bottlenecks'' linking adjacent spores.

Pore-Size Distribution ≤1 mm EPD

Water desorption, using soil cores, has wide application for the determination of pore-size distribution. The method is suitable for undisturbed cores containing relatively small structural units (<2 cm diameter) and for remolded cores. This method is based on the determination of the water desorption curve (moisture characteristic). The curve relates soil moisture content to the energy status of soil water (water potential). The water potential is expressed in terms of matric potential. Based on the assumptions of the capillary model, the volume of water removed from the soil in response to a change in matric potential corresponds to the change in energy status of the soil water and the volume of pores above a specific diameter. Mathematically, this is expressed by the Kelvin equation:

$$d = \frac{4\gamma \cos \alpha}{pgh} \tag{57.16}$$

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where d is the diameter (m) of the largest pores that remain full of water after a matric potential (h) in meters of water is applied; γ is the surface tension of water (72.75 mJ m^{-2} at 20°C); α is the contact angle of the water held in the pore (usually taken to be zero); p is the density of water (0.998 Mg m^{-3} at 20°C); and g is the acceleration due to gravity (9.8 m s^{-2}). Equation 57.16 indicates that pores that can maintain a water meniscus against the combined force of pgh have an upper limiting radius. Therefore, applying a negative potential of −1 m of water (equivalent to −0.1 bar or −10 kPa matric potential) will give a diameter of 30 μm at 20°C for the largest pores full of water.

Use of the above procedure, however, requires that decreasing matric potential progressively drains the soil. Otherwise, soil hysteresis influences water content at a given potential. The pore-size distribution must also remain stable during determination of the water desorption curve.

In fine-textured soils (clay content above 30%), shrinkage can change the pore-size distribution over time, whereas in very sandy soils (sand content above 80%), low water adhesion causes gravitational loss of water. Under these conditions, other techniques to determine pore-size distribution such as mercury intrusion and nitrogen sorption have been developed. Both of these methods are based on the cylindrical pore model for pore-size distribution. Danielson and Sutherland (1986) and Flint and Flint (2002) provide details on the mercury intrusion method. The water desorption method described below is best suited for medium textured soils and for both sandy and fine-textured soils. However, the method is applicable in clay soils at relatively high matric potentials (0 to −1 kPa). Generally, the water desorption method is used at low potentials (−1 to −100 kPa) where Equation 57.16 is most applicable.

Methods to determine water desorption between 0 and −20 kPa matric potential use tension table water extraction (Topp and Zebchuk 1979; Ball and Hunter 1988; Romano et al. 2002). Methods to determine water desorption at lower than −20 kPa potential require the use of pressure plate extraction (20–500 kPa) and pressure membrane extraction (100–1500 kPa). These three extraction methods and procedures are described in Chapter 72.

Calculations

To calculate the water desorption curve, plot volumetric moisture content (θ) versus matric potential. Determine volumetric soil moisture content (%) at each matric potential as follows:

$$\theta_v = \frac{W_p - W_2}{V_t} \times 100/D_w \tag{57.17}$$

where W_p is the weight of soil plus core at a specific matric potential and W_2 is the weight of dry soil plus core. Determination of pore-size distribution is based on the information given in Equation 57.17, where the diameter of pores remaining full of water was 30 μm after equilibration at a potential of −1 m (−10 kPa). Thus, the diameter of the smallest pore drained at a specific suction would be

$$D\ (\mu m) = \frac{300}{kPa} \tag{57.18}$$

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Calculation of the volume of water removed between two specific pore-size diameters would equal the volume of pore space for that pore-size range.

Comments

1. The pore-size diameters are approximate and therefore designated as EPD. In addition, the contact angle (α) in Equation 57.16 is assumed to be zero; this may not always be correct. Further, the method provides no information on pore continuity or shape. Also, for soil cores with a relatively large volume of macropores (EPD >50 μm), it is difficult to obtain complete saturation by capillary wetting. Under these circumstances, use of vacuum wetting (Ball and Hunter 1988) or estimation of total porosity from bulk density using Equation 57.12 is advised.

2. Use of undisturbed soil cores will prove a problem on recently cultivated soils due to loose cores. Generally some degree of compaction by weathering must occur before intact cores can be obtained.

3. During the saturation procedure, formaldehyde solution (4% w/v) can be sprayed on the surface of the core to remove or suppress the activity of earthworms and other macrofauna (Ball and Hunter 1988).

Pore-Size Distribution $\geq$1 mm EPD

The size and continuity of pores $>$1 mm EPD are particularly important for environmental modeling of infiltration of water and solute solution. Douglas (1986) describes morphological procedures for assessing the dimensions of large macropores. Procedure 1 is recommended for soil that does not shrink measurably on drying. Procedure 2 is recommended for soil that remains stable during saturation.

Procedure 1

Oven dry the core sample. Record the number and diameter of channel-type pores of diameter $\geq$1 mm judged to have openings at both ends of the sample. Measure pore diameter using a set of 1–8 mm rigid aluminium rod gauges.

Douglas (1986) also proposed a method of identification of functional macropores, i.e., those responsible for flow-through samples.

Procedure 2

Saturate the core sample. Pour a solution of Rhodamine-B dye through the samples. After drainage, extrude the lower outflow ends of the cores from their retaining rings for a distance of 1 cm and carefully pick off the extruded 1 cm of soil. The continuous, stained pores thus exposed are traced on to acetate sheets, counted, and measured.

Pore Size and Shape Using Computer Image-Analysis Techniques

Image-analysis techniques not only provide pore-size distribution but also the pore shapes, and could give three-dimensional results. An alternative to staining or direct

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measurement techniques is x-ray CT (Rasiah and Aylmore 1998; Gantzer and Anderson 2002). In this technique, CT scanners are used to scan relative pixel density in the undisturbed cores and image-processing software is used to calculate the area, volumes, and three-dimensional measurements (Perret et al. 1999). Another technique is image analysis of soil porosity using difference imagery of stereo photographs (Grevers and De Jong 1990; Sort and Alcańiz 1999).

57.4.6 Pore Continuity or Function Indices

The continuity of soil macropores is particularly important in determining the conductivity of soil for gas movement, water infiltration, and root exploration. Indices of continuity measure the ability of the soil spores to conduct fluid. Gas is commonly the fluid used, because it does not change the soil structure when it diffuses or flows through the soil. Use of gas movement allows measurements to be repeated at different soil moisture contents. This allows assessment of the change of pore continuity with air-filled porosity.

In order to measure pore continuity, measure gas relative diffusivity (D/D_o) and air permeability (K_a) (in units of μm^2) and air-filled porosity (F_a) (volume percentage) in soil cores in the laboratory. Techniques for measuring K_a are given in Chapter 61. In addition, Ball and Smith (1991) present an extensive review of suitable techniques.

In terms of gas relative diffusivity, pore continuity C_d is

$$C_d = \frac{D/D_o}{F_a} \times 100 \tag{57.19}$$

This factor, C_d, derived by Ball (1981b), ranges from 0 for completely blocked pores to 1 for straight tubular pores aligned with the direction of diffusing gas. Schjonning et al. (2002) discuss how large values of C_d can also be interpreted as indicating a large number of marginal pores lying off the arterial pathways for diffusion, a concept of pore function suggested by Arah and Ball (1994).

In terms of air permeability, pore continuity C_k or macropore organization (Groenevelt et al. 1984; Blackwell et al. 1990) is

$$C_k = \frac{K_a}{F_a} \times 100 \tag{57.20}$$

Other indices of pore continuity and pore tortuosity were reviewed by Ball et al. (1988).

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Chapter 58
Soil Consistency: Upper and Lower Plastic Limits

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58.1 INTRODUCTION

The (Atterberg) consistency limits of soils are used primarily in classifying cohesive soil materials for engineering purposes (ASTM 2000a), and are strongly correlated to other fundamental soil properties (De Jong et al. 1990). They are also used widely in the estimation of other test indices useful for soil engineering interpretations, such as shear strength and bearing capacity, compressibility, swelling potential, and specific surface (reviewed in McBride 1989).

As gravimetric water contents, the shrinkage limit, lower plastic limit (w_P), and upper plastic (liquid) limit (w_L) test indices represent the three major points of transition in soil consistency amongst the solid, semisolid, plastic, and liquid states, respectively. The standard American Society for Testing and Materials (ASTM) test procedures for w_L and w_P determination (ASTM 2000b) are somewhat subjective and arbitrary shear tests, and so are prone to significant operator and mechanical variability. Test reproducibility has not been improved with the introduction of the one-point w_L test method, which reduces the testing time (and cost) as compared to the normally iterative procedures.

Considerable effort has been directed at researching alternative and procedurally unified test methods, including desorption by pressure plate extraction (reviewed in McBride 1989), consolidation of soil–water suspensions (McBride and Bober 1989; McBride and Baumgartner 1992), measurement of paste viscosity, and drop-cone penetration. Some methods have shown very promising results, but only w_L determination by cone penetration has been standardized to date, and is the preferred method of the British Standards Institution (BS 1377:Part 2:1990) (BSI 2000). It appears unlikely, however, that a direct measurement of w_P by the cone penetration method is possible (Harison 1988).

This chapter outlines only those test protocols that have been standardized and that are widely accepted and used. The primary sources for these procedures were AASHTO (2000a,b) ASTM (2000b), BSI (2000), and Sheldrick (1984).

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58.2 UPPER PLASTIC (LIQUID) LIMIT

The upper plastic (liquid) limit of a cohesive soil is defined as the gravimetric water content (percentage) corresponding to an arbitrary limit between the liquid and plastic states of consistence when in a remolded condition. In accordance with the standard ASTM (Casagrande) test procedure, it is the water content at which a pat of soil, cut by a standard-sized groove, will flow together for a distance of 13 mm under the impact of 25 blows in a standard ASTM liquid limit device. The undrained cohesion of soils in this consistency state is approximately 1.7 kPa (Wroth and Wood 1978).

58.2.1 Casagrande Method (ASTM D4318-98; AASHTO T89-90; BS 1377:Part 2:1990)

Apparatus and Materials

1. ASTM liquid limit device with grooving tool
2. Metal spatula (7–8 cm in length, 2 cm wide)
3. Evaporating dish (10–12 cm in diameter)
4. Soil sample containers for water content measurement
5. Balance (sensitive to 0.01 g)
6. Drying oven (105°C)
7. Air-dry soil sample of about 100 g and passing a No. 40 (425 μm) sieve

Procedure

1. Place the soil sample in the evaporating dish and thoroughly mix with 15 to 20 mL of distilled water by alternately and repeatedly stirring, kneading, and chopping with a spatula. Make further additions of water in increments of 1 to 3 mL. Thoroughly mix each increment of water with the soil, as previously described, before adding another increment of water.

2. When sufficient water has been thoroughly mixed with the soil to produce a consistency that will require 30 to 35 drops of the cup to cause closure, place a portion of the mixture in the cup above the spot where the cup rests on the base, and squeeze it down and spread it into position with as few strokes of the spatula as possible. Care should be taken to prevent the entrapment of air bubbles within the soil mass. With the spatula, level the soil and at the same time trim it to a depth of 1 cm at the point of maximum thickness. Return the excess soil to the evaporating dish. Divide the soil in the cup by firm strokes of the grooving tool along the diameter through the centerline of the cam follower so that a clean, sharp groove of the proper dimensions will be formed. To avoid tearing of the sides of the groove or slipping of the soil pat on the cup, up to six strokes, from front to back or from back to front counting as one stroke, shall be permitted.

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Each stroke should penetrate a little deeper until the last stroke from back to front scrapes the bottom of the cup clean. Make the groove with as few strokes as possible.

3 Lift and drop the cup by turning the crank at the rate of 2 rps, until the two halves of the soil pat come in contact at the bottom of the groove along a distance of about 13 mm. Record the number of drops (N) required to close the groove along a distance of 13 mm.

4 Remove a slice of soil approximately the width of the spatula, extending from edge to edge of the soil pat at right angles to the groove and including that portion of the groove in which the soil flowed together, and place in a suitable tared container. Determine the gravimetric water content.

5 Transfer the soil remaining in the cup to the evaporating dish. Wash and dry the cup and grooving tool, and reattach the cup to the carriage in preparation for the next trial.

6 Repeat steps 2 to 5 for at least two additional trials, with the soil collected in the evaporating dish, to which sufficient water has been added to bring the soil to a more fluid condition. The object of this procedure is to obtain samples of such consistency that the number of drops required to close the groove will be above and below 25. The number of drops should be less than 35 and exceed 15. The test shall always proceed from the drier to the wetter condition of the soil.

Calculations

1 Calculate the gravimetric water content of the soil (w), expressed as a percentage of water in the sample on a dry mass basis (%kg kg^{-1}), as follows:

$$w = \left(\frac{\text{mass of water}}{\text{mass of oven-dry soil}}\right) \times 100 \tag{58.1}$$

2 Plot a flow curve representing the relationship between gravimetric water content and corresponding numbers of drops of the cup on a semilogarithmic graph with w as abscissa on the linear scale, and the number of drops (N) as ordinate on the logarithmic scale. The flow curve is a straight line drawn as nearly as possible through the three or more plotted points.

3 Take the water content corresponding to the intersection of the flow curve with the $N = 25$ ordinate as the upper plastic limit (liquid limit) of the soil. Report the w_L test index to the nearest whole number.

Comments

1 Before testing, inspect the liquid limit device to determine that the device is in good working order, that the pin connecting the cup is not worn sufficiently to permit side play, that the screws connecting the cup to the hanger arm are tight, and that a groove has not been worn in the cup through long usage. Also ensure that the dimensions of the grooving tool are to specification (ASTM 2000b).

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2 By means of the gauge on the handle of the grooving tool and the adjustment plate, adjust the height to which the cup is lifted so that the point on the cup that comes in contact with the base is exactly 1 cm above the base. Secure the adjustment plate by tightening the screws. With the gauge still in place, check the adjustment by revolving the crank rapidly several times. If the adjustment is correct, a slight ringing sound will be heard when the cam strikes the cam follower. If the cup is raised off the gauge or no sound is heard, make further adjustments.

3 A motorized version of the liquid limit device with a blow (revolution) counter is commercially available and should be used if possible to minimize operator variability.

58.2.2 One-Point Casagrande Method (ASTM D4318-98; AASHTO T89-90; BS 1377:Part 2:1990)

Procedure

1 This single-point method was based on a study involving regression analysis of a large number of tested soils, and can be used when the available sample size is small or when only an approximation of w_L is needed. The requirements for the apparatus, the soil sample preparation, and the mechanical device adjustments are identical to those under Section 58.2.1.

2 Proceed in accordance with procedural steps 1 through 5 under Section Procedure, p. 762, except that a water content sample shall be taken only for the accepted trial. The accepted trial requires between 20 and 30 drops of the cup to close the groove and at least two consistent consecutive closures are to be observed before taking the water content sample for calculation of the upper plastic limit. The test should always proceed from the drier to the wetter condition of the soil.

Calculations

1 Calculate the percent gravimetric water content (w) of the soil for the accepted trial as per Equation (58.1).

2 Determine the upper plastic limit using the following formula:

$$w_L = w(N/25)^{0.12} \tag{58.2}$$

where N is the number of drops of the cup required to close the groove at the test water content.

3 Report the w_L test index to the nearest whole number.

58.2.3 Drop-Cone Penetrometer Method (BS 1377:Part 2:1990)

Apparatus and Materials

1 Standard drop-cone penetrometer.

2 Cone of stainless steel or duralumin approximately 35 mm long, with a smooth, polished surface and an angle of 30 $\pm$ 1°. The mass of the cone together with its sliding shaft is 80.00 $\pm$ 0.05 g.

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3 Noncorrodible airtight container.

4 Metal cup approximately 5.5 cm in diameter and 4.0 cm deep with rim parallel to the flat base.

5 Metal spatula (7–8 cm in length, 2 cm wide).

6 Evaporating dish (10–12 cm in diameter).

7 Soil sample containers for water content measurement.

8 Balance (sensitive to 0.01 g).

9 Drying oven (105°C).

10 Air-dry soil sample of about 200 g and passing a No. 40 (425 μm) sieve.

Procedure

1 A sample weighing at least 200 g is placed on the evaporating dish and mixed thoroughly with distilled water using the spatula until the mass becomes a thick homogeneous paste. This paste is then allowed to stand in the airtight container for about 24 h to allow the water to permeate throughout the soil mass.

2 The sample is then removed from the container and remixed for at least 10 min. If necessary, further water is added so that the first cone penetration reading is approximately 15 mm.

3 The remixed soil is pushed into the cup with a spatula, taking care not to trap air. The excess soil is removed to give a smooth surface. The cone is lowered so that it just touches the surface of the soil. When the cone is in the correct position, a slight movement of the cup will leave a slight mark on the surface of the soil and the reading of the dial gauge is noted to the nearest 0.1 mm. The cone is then released for a period of 5 ± 1 s. If the apparatus is not fitted with an automatic release and locking device, care should be taken to isolate the apparatus from disturbance during these operations. After the cone has been locked in position, the dial gauge is lowered to the new position of the cone shaft and the reading noted to the nearest 0.1 mm. The difference between the readings at the beginning and end of the test is recorded as the depth of cone penetration.

4 The cone is lifted out and cleaned carefully. A little more wet soil is added to the cup and the process repeated. If the difference between the first and second penetration readings is <0.5 mm, the average of the two penetrations is recorded. If the second penetration is >0.5 mm and <1 mm different from the first, a third test shall be carried out. If the overall range is then <1 mm, a water content sample (about 10 g) is taken from the area penetrated by the cone and the water content determined. The average of the three penetrations is recorded. If the overall range is >1 mm, the soil shall be removed from the cup, remixed, and the test repeated until consistent results are obtained.

5 The operations described in steps 3 and 4 are to be repeated at least four times using the same sample to which further increments of distilled water have been added.

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The amount of water added shall be chosen so that a range of penetration values of approximately 15 to 25 mm is covered.

Calculations

The relationship between the gravimetric water content and the depth of cone penetration is plotted with the percentage water content as abscissa and the cone penetration as ordinate, both on linear scales. The best straight line fitting the plotted points is drawn through them. The water content corresponding to a cone penetration of 20 mm is taken as the upper plastic limit of the soil and is expressed to the nearest whole number. The method of obtaining the w_L shall be stated (i.e., using the cone penetrometer).

Comments

A version of the drop-cone penetrometer equipped with a digital automatic controller (and direct readout) is commercially available and should be used if possible to minimize operator variability. The 20 mm penetration depth standard should be used with caution, as many studies outside of Britain (including Canada) have documented both under- and over-estimation of the Casagrande w_L using the cone penetration method (cf. McBride and Baumgartner 1992; Leroueil and Le Bihan 1996).

58.3 LOWER PLASTIC LIMIT

The lower plastic limit of a cohesive soil is defined as the gravimetric water content (percentage) corresponding to an arbitrary limit between the plastic and semisolid states of consistence when in a remolded condition. In accordance with the standard ASTM (Casagrande) test procedure, it is the water content at which a soil will just begin to crumble when rolled into a thread approximately 3.2 mm in diameter. The undrained cohesion of soils in this consistency state is approximately 170 kPa (Wroth and Wood 1978).

58.3.1 Casagrande Method (ASTM D4318-98; AASHTO T90-87; BS 1377:Part 2:1990)

Apparatus and Materials

1. Evaporating dish (10–12 cm in diameter)
2. Metal spatula (7–8 cm in length, 2 cm wide)
3. Surface for rolling (e.g., a ground-glass plate)
4. Soil sample containers for water content measurement
5. Balance (sensitive to 0.01 g)
6. Drying oven (105°C)
7. Air-dry soil sample of about 15 g and passing a No. 40 (425 μm) sieve

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Procedure

1 If the lower plastic limit only is required, take about 15 g of air-dried soil, place in an evaporating dish and thoroughly mix with distilled water until the mass becomes plastic enough to be easily shaped into a ball. Take a portion of this ball weighing about 8 g for the test sample.

2 If both the upper and lower plastic limits are required, take a test sample weighing about 8 g from the thoroughly wet and mixed portion of the soil prepared for the liquid limit test (see Section 58.2.1). Take the sample at any stage of the mixing process at which the mass becomes plastic enough to be easily shaped into a ball without sticking to the fingers excessively when squeezed.

3 Squeeze and form the 8 g test sample taken in accordance with steps 1 or 2 into an ellipsoidal-shaped mass. Roll this mass between the fingers and the ground-glass plate lying on a smooth horizontal surface with just sufficient pressure to roll the mass into a thread of uniform diameter throughout its length. The rate of rolling shall be between 80 and 90 strokes per min, counting a stroke as one complete motion of the hand forward and back to the starting position again.

4 When the diameter of the thread becomes about 3.2 mm, break the thread into six or eight pieces. Squeeze the pieces together between the thumbs and fingers of both hands into a uniform mass roughly ellipsoidal in shape, and reroll. Continue this alternate rolling to a thread 3.2 mm in diameter, gathering together, kneading, and rerolling, until the thread crumbles under the pressure required for rolling and the soil can no longer be rolled into a thread. The crumbling may occur when the thread has a diameter greater than 3.2 mm. This shall be considered a satisfactory end point, provided the soil has been previously rolled into a thread 3.2 mm in diameter. The crumbling will manifest itself differently with the various types of soil. Some soils fall apart in numerous small aggregations of particles; others may form an outside tubular layer that starts splitting at both ends. The splitting progresses toward the middle, and finally, the thread falls apart in many small platy particles. Heavy clay soils require much pressure to deform the thread, particularly as they approach the lower plastic limit, and finally, the thread breaks into a series of barrel-shaped segments each about 6.4 to 9.5 mm in length. The operator should not attempt to produce failure at exactly 3.2 mm diameter by allowing the thread to reach 3.2 mm, then reducing the rate of rolling or the hand pressure, or both, and continuing the rolling without further deformation until the thread falls apart. It is permissible, however, to reduce the total amount of deformation for marginally plastic soils by making the initial diameter of the ellipsoidal-shaped mass nearer to the required 3.2 mm final diameter.

5 Gather the portions of the crumbled soil together and place in a suitable tared container. Determine the gravimetric water content.

Calculations

1 Calculate the percent gravimetric water content of the remolded soil as per Equation (58.1). Report this value as the w_P test index to the nearest whole number.

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2 Calculate the plasticity index of a soil as the difference between its upper and lower plastic limits, as follows:

$$\text{Plasticity index} = w_L - w_P \tag{58.3}$$

3 Report the difference calculated in Equation (58.3) as the plasticity index, except under the following conditions:

(i) When the w_L or w_P test indices cannot be determined, report the plasticity index as NP (nonplastic).

(ii) When the soil is extremely sandy, the w_P test is to be performed before the w_L test. If the w_P cannot be determined, report the plasticity index as NP.

(iii) When the w_P is greater than or equal to w_L, report the plasticity index as NP.

Comments

Test reproducibility and rapidity can be improved by using a soil rolling device of the sort proposed by Bobrowski and Griekspoor (1992) without significantly deviating from the standard manual methods.

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Chapter 59
Compaction and Compressibility

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59.1 INTRODUCTION

The passage of agricultural and forest machines over soil is the main cause of compaction of cultivated and forest soils. If the load applied during trafficking is larger than the resistance of the soil against compaction (mechanical soil strength), compaction of the soil occurs. Compaction (also referred to as compression) is a reduction of the volume of a given mass of soil. The decrease in soil volume is due to a decrease in the volume of pore space through partial expulsion of soil air and water. When soil is compacted, the porosity is decreased and, conversely, the bulk density is increased. The decrease in porosity due to compaction is accompanied by a modification of the pore geometry, i.e., pore morphology and connectivity. Hence, soil compaction modifies soil structure. Pore geometry, which may also be strongly affected by shearing, is a very important component of soil structure because it greatly affects the properties of water and gas transport in soil.

Soil compaction intensity depends on vehicle and soil characteristics through:

- The stress applied on the soil surface (i.e., at the soil–tire or soil–track interface) and the area of contact between the soil and the tire or track that is determined by loading characteristics (load, tire type, dimension and inflation pressure, track properties) and soil conditions.

- The mechanical soil strength, i.e., the soil resistance against the decrease in soil volume. It is affected by soil type and soil conditions (soil texture, soil organic matter content, soil structure, bulk density, and soil moisture status). For a given

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texture, the mechanical soil strength is strongly influenced by soil moisture and bulk density. Generally, soils become stronger with decreasing moisture content and increasing density.

The strength of a soil is obtained from the compressive behavior of soil, which can be measured in a so-called compression test in the laboratory or in the field. The compressive behavior of soil is expressed graphically in the relationship between the logarithm of applied stress and some parameter related to the packing state of the soil, e.g., strain, void ratio, specific volume, or bulk density. This relationship is also referred to as the compression curve.

An idealized soil compression curve is shown in Figure 59.1. Two domains can be defined: a swelling line (SL) (also referred to as recompression line) at a lower stress range and a virgin compression line (VCL) at higher stresses. Both lines are separated by a critical stress, termed the precompression stress (also referred to as preconsolidation stress or preload).

Soil behavior is believed to be mainly elastic in the recompression range and mainly plastic in the virgin compression range. The precompression stress is widely used to characterize the soil-bearing capacity, i.e., the maximum stress that can be applied during trafficking that results in only limited soil compaction.

The slope of the SL is called swelling (or recompression) index, C_s, while the slope of the VCL is termed compression index, C_c (Figure 59.1).

Consequently, three parameters are required to describe the compressive behavior of soil: the precompression stress, σ_p, the compression index, C_c, and the swelling index, C_s.

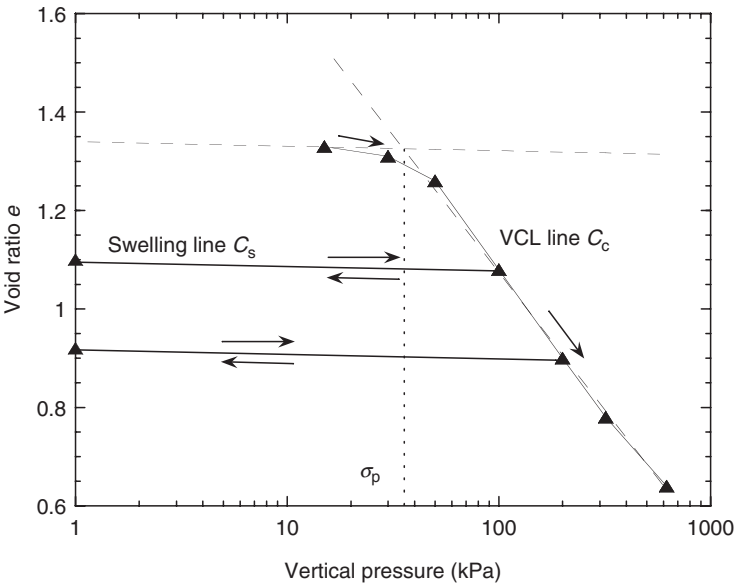

FIGURE 59.1. Stress–strain relationship for a Eutric Cambisol at an initial gravimetric water content of 0.15 g g^{-1} and an initial void ratio, e_0, of 1.34. The swelling index (C_s) was calculated to be 0.009 and the compression index (C_c) to be 0.51. The precompression stress, σ_p, was 36 kPa with the Casagrande method.

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These parameters are required input parameters in soil compaction models (Défossez and Richard 2002).

Stress–strain relationships of soils are measured in laboratory and field tests to obtain σ_p, C_c, and C_s. In order to characterize soil compressibility, two easily applicable and commonly used methods are presented here: (1) a laboratory method using the oedometer apparatus and (2) a field method using the plate sinkage apparatus. This is followed by a section on how to determine the precompression stress.

59.2 ONE-DIMENSIONAL LABORATORY TEST: OEDOMETER

The oedometer test is a uniaxial, confined compression test. It simulates vertical loading applied to soil in the field by a tire. The basic setup of the oedometer is shown in Figure 59.2. The soil sample, which is confined in a stiff ring, is loaded uniaxially in a vertical direction. The stiff ring prevents the sample from lateral deformation. During the compression test, axial stress is applied and the resulting soil displacement is measured.

59.2.1 Apparatus

The oedometer apparatus contains a loading device, a compression cell, and devices to record the applied stress and the resulting displacement.

1. Loading device: a uniaxial stress in vertical direction is applied to the soil specimen either by dead weights, by pneumatic methods, or by a step-motor.

2. Compression cell: the cell is usually a circular metal ring (75 mm diameter, 15–20 mm height), which encloses the soil between two porous plates. The ratio of diameter to height should be greater than 3 to minimize the influence of side-wall friction along the cylinder walls.

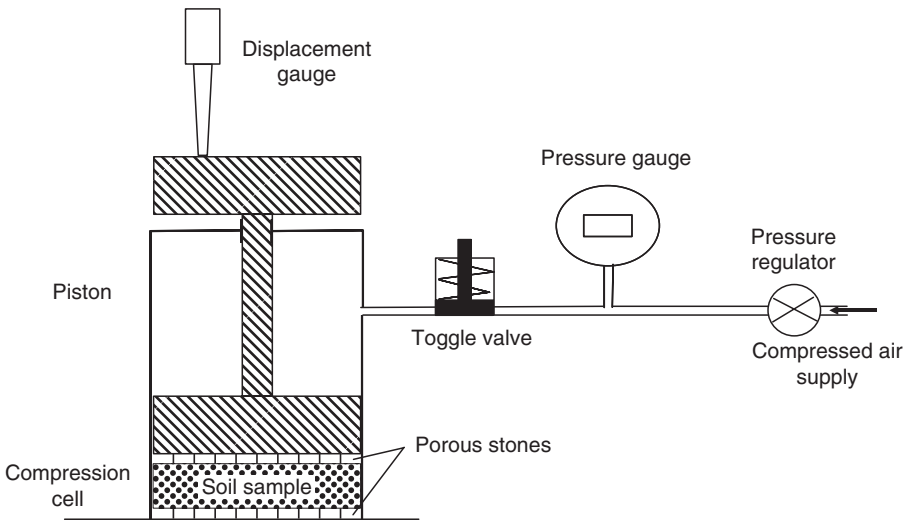

FIGURE 59.2. Oedometer apparatus using a pneumatic loading device.

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3 Gauge for measuring applied force or stress (stress is calculated from the force by dividing the applied force by the surface area of the soil sample).

4 Displacement transducer with a range of e.g., 15 mm and an accuracy of 0.002 mm.

5 Optional: an electronic data acquisition system for recording displacement and stress data.

59.2.2 Preparation of Soil Samples

Tests may be performed on intact/undisturbed or on remolded soil samples. Preparation for the latter is described here.

Remolded soil samples are usually prepared from sieved soil. Air-dry soil is sieved through a mesh with a diameter of 2 mm. The gravimetric soil water content of the air-dry soil, w_{airdry}, is determined (see Section ''Soil Water Analysis''). Then, the soil is wetted to the desired soil water content by placing a plastic box containing a mass of air-dry soil, m_{airdry}, on a balance and adding water with a spray. A period of 24 h is allowed for water redistribution.

The mass of water, ${m_{\text{w}}}^{\text{added}}$, which has to be added to the soil in order to obtain the desired gravimetric soil water content, w_{desired}, is

$${m_{\text{w}}}^{\text{added}} = m_{\text{airdry}} \frac{(w_{\text{desired}} - w_{\text{airdry}})}{(w_{\text{airdry}} + 1)} \tag{59.1}$$

A soil core at a desired bulk density, ρ_{desired}, is prepared by packing the following mass of soil, m_{soil}, in the compression cell

$$m_{\text{soil}} = \rho_{\text{desired}} V_{\text{cylinder}} \tag{59.2}$$

where V_{cylinder} is the volume of the oedometer cylinder.

59.2.3 Compression Tests

Soil samples are loaded either sequentially or at constant displacement velocity. Sequential loading (or step loading) is widely used.

Sequential Loading

Sequential loading implies that stress is applied stepwise. Each stress level is hereby applied for a certain loading time. The loading time may be chosen depending upon soil properties and objective of the test. It is typically 30 min in agricultural soil mechanics research and 24 h in geotechnical engineering. However, longer or shorter loading times may be chosen for different reasons. A typical sequence of applied stress levels is 15, 30, 50, 100, 200, 300, and 600 kPa.

Procedure

1 Weigh the soil sample before starting the compression test.

2 Place the cylinder in the oedometer apparatus.

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3 Apply stress corresponding to the first stress level and keep this stress constant for the duration of the desired loading time per stress level.

4 After loading for exactly the desired loading time per stress level, measure the displacement.

5 Apply the stress corresponding to the next stress level.

6 Repeat steps 4 and 5 until the last stress level is reached.

7 After removing the cylinder from the oedometer, weigh the sample.

8 Dry the sample in an oven at 105°C for at least 24 h and weigh.

Alternatively, the soil sample may be completely unloaded between steps 4 and 5.

Stress and displacement data may be recorded with high resolution during the compression test. This requires an electronic data acquisition system.

Loading at Constant Displacement Velocity

Loading at constant displacement velocity implies that the piston of the oedometer apparatus (see Figure 59.2) is moving downward at a constant velocity, and the corresponding stress is measured. Measuring at constant displacement velocity requires an electronic data acquisition system for recording displacement and stress data. The procedure is as explained earlier. Instead of steps 3–6, the soil sample is loaded at constant displacement velocity and displacement–stress data are recorded with a data logger.

59.2.4 Calculations

Initial conditions are determined for each specimen. The initial water content, w_0, is calculated as

$$w_0 = \frac{m_0 - m_{\text{dry}}}{m_{\text{dry}}} \tag{59.3}$$

where m_0 is the mass of the soil sample at initial moisture conditions before the compression test and m_{dry} the mass of the oven-dried sample. The initial void ratio, e_0, is calculated as

$$e_0 = \frac{\rho_s}{\rho_0} - 1 = \frac{\rho_s V_0}{m_s} - 1 \tag{59.4}$$

where ρ_s is the density of solids, ρ_0 the initial dry bulk density, and V_0 the initial sample volume (i.e., the volume of the oedometer cylinder).

59.2.5 Presentation of Results

The results of a compression test are presented graphically as the relationship between the logarithm of the applied stress and some parameter related to the packing state of the soil,

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e.g., strain, bulk density, or void ratio, as shown in Figure 59.1. Void ratio, e, is calculated from the measured displacement, Δh, as

$$e(\Delta h) = \frac{\rho_s V(\Delta h)}{m_s} - 1 = \frac{\rho_s \pi r^2 (h_0 - \Delta h)}{m_s} - 1 \tag{59.5}$$

where V, r, and h_0 are the volume, radius, and initial height of the cylindrical soil sample, respectively.

From the compression curve (Figure 59.1), C_s, C_c, and σ_p are obtained. The compression index, C_c, i.e., the slope of the VCL, and the swelling index, C_s, i.e., the slope of the SL, are calculated by

$$C_c = \frac{\Delta e}{\log(\Delta\sigma)}; \quad \sigma \in (\text{VCL}) \tag{59.6}$$

$$C_s = \frac{\Delta e}{\log(\Delta\sigma)}; \quad \sigma \in (\text{SL}) \tag{59.7}$$

where e is the void ratio and σ the applied normal stress. Determination of σ_p is explained in detail in Section 59.4.

59.3 *IN SITU* PLATE SINKAGE FIELD TEST: PLATE PENETROMETER

In situ plate sinkage tests are an established procedure used in the prediction of rolling resistance for off-road vehicles. Alexandrou and Earl (1995) showed that the plate sinkage test can be applied for determining soil precompression stress.

An example of a plate penetrometer is shown in Figure 59.3. The plate penetrometer contains a load cell to measure the required force, a depth potentiometer to measure the sinkage of the circular plate, and an electrical motor, which drives the plate downward at a desired plate sinkage (displacement) velocity.

59.3.1 PROCEDURE

1. Excavate the soil to the desired depth (the soil may be subjected to compression at the soil surface, or at any desired depth). Note that the area of excavated soil has to be large enough to place the apparatus within it, and to potentially allow for several measurements (replicates) at the same depth.

2. Level the soil surface at the desired depth in order to allow the penetrometer plate to be driven downward in an exactly vertical direction. When conducting measurements at the soil surface, crop residues should be removed.

3. Put the apparatus in place. The apparatus shown in Figure 59.3 needs two persons acting as counterweights, standing on the footplates on either side of the apparatus.

4. Start the plate sinkage test.

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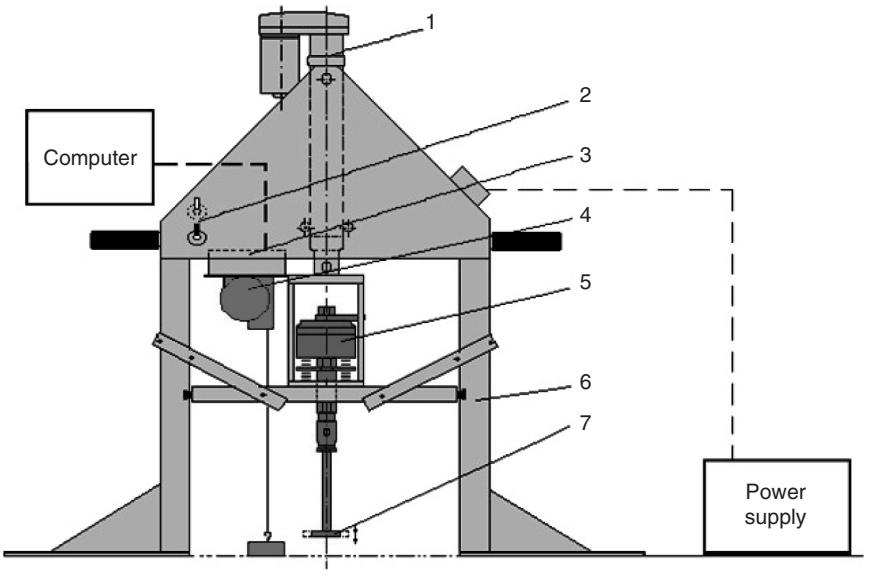

FIGURE 59.3. Side view of a plate penetrometer: (1) electric power unit; (2) switch to record data; (3) data shuttle; (4) depth sensor; (5) load sensor; (6) frame; (7) penetration plate. (From Dawidowski, J.B., Morrison, J.E., and Snieg, M., *Trans. Am. Soc. Agr. Eng.*, 44, 1059, 2001. With permission.)

5 During each test, the vertical normal force and the sinkage (vertical displacement) are recorded (by a data logger) with high resolution (e.g., 100 Hz).

The apparatus used by Dawidowski et al. (2001) and Keller et al. (2004) had the following dimensions:

1 Plate diameter: 49 mm

2 Maximum loading: 4400 N, with a maximum error of $\pm 0.05\%$

3 Maximum error of the depth penetrometer: $\pm 0.1\%$

4 Maximum stroke of the plate: 200 mm

5 Downward velocity of the plate: 7 or 25 mm s^{-1}

6 Maximum frequency of data logging: 1000 Hz

59.3.2 Calculations and Presentation of Results

The recorded data can be plotted in a log σ–displacement diagram, from which the precompression stress, swelling index, and compression index can be obtained as described in Section 59.2.4 and Section 59.2.5.

Note that there is no control over lateral deformations below the penetrometer plate in a plate sinkage test. Therefore, the compression curve obtained from the plate sinkage test is

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expressed in a log σ–displacement diagram (whereas the compaction curve from the oedometer test is normally expressed in a log σ–e diagram). Therefore, the expression ''compression index'' is physically inaccurate when applied to the plate sinkage test. However, lateral deformations should be small for small displacements (see also Section 59.5.1).

59.4 DETERMINATION OF THE PRECOMPRESSION STRESS

There are several procedures known for estimating the precompression stress from the compression curve. Some of the most commonly used methods are given in Dias and Pierce (1995).

59.4.1 Casagrande's Method

The procedure developed by Casagrande (1936) is considered as a standard method. The method was developed empirically from a large number of tests on different types of soils.

The procedure consists of six steps (Figure 59.4):

1. Determine the position of the VCL with a sufficient number of data points.
2. Determine the point, *T*, that corresponds to the smallest radius of curvature.
3. Draw a tangent, *t*, to the curve through point *T*.
4. Draw a horizontal line, *h*, through point *T*.
5. Draw the bisecting line, *b*, between the tangent and the horizontal through point *T*.
6. The precompression stress, σ_p, is found as the point of intersection of the bisecting line and the VCL.

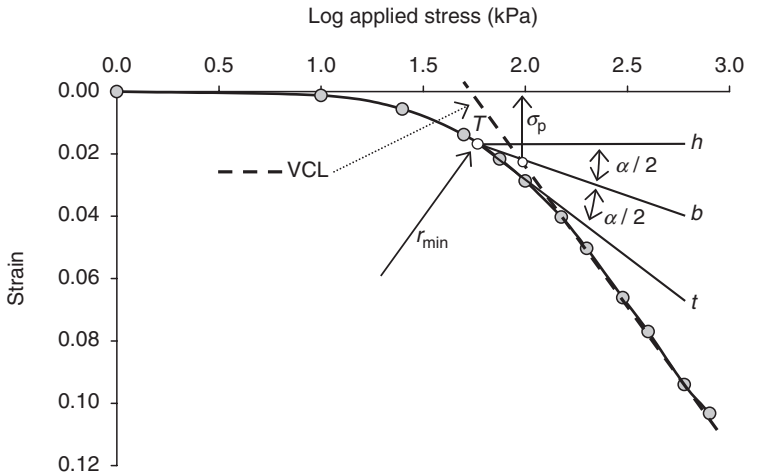

FIGURE 59.4. Determination of the precompression stress according to Casagrande. The procedure and the symbols are described in the text. (Adapted from Casagrande, A., *Proceedings of the International Conference on Soil Mechanics and Foundation Engineering*, Vol. III, Cambridge, UK, 1936.)

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The Casagrande procedure is originally a graphical procedure, i.e., the point corresponding to the smallest radius of curvature is determined by visual observation. However, the visual determination is subjective and scale-dependent. Dawidowski and Koolen (1994) developed a mathematical procedure to calculate precompression stress according to Casagrande. Another possibility is to fit the data points to a mathematical equation, from which the above mentioned points 1–6 can be calculated. The data points may be fitted to a logarithmic function (Bailey et al. 1986) to the van Genuchten equation (Baumgartl and Köck 2004) or to a polynomial equation (Arvidsson and Keller 2004).

59.4.2 Other Methods

As the determination of the point that corresponds to the smallest radius of curvature may be difficult, other procedures generally involving regression analysis have been developed for the determination of the precompression stress (Sällfors 1975; Culley and Larson 1987; Jose et al. 1989; Lebert and Horn 1991; Dias and Pierce 1995).

The easiest method is to estimate precompression stress as the intersection of two lines: (1) SL, i.e., the regression line obtained for the points of applied stress sequence in the recompression portion of the compression curve and (2) VCL, i.e., the regression line obtained for the points in the virgin compression portion of the compression curve (Dias and Pierce 1995).

Note that different determination procedures can result in slightly different values for the precompression stress (Arvidsson and Keller 2004).

59.5 General Comments

59.5.1 Comparison between Confined Laboratory Test and Field Assessment of Soil Compressibility

In an oedometer test (Section 59.2), the lateral (i.e., horizontal) strain is fully prevented by a cylindrical stiff ring in which the sample is enclosed. The plate sinkage test in the field (Section 59.3) takes place in only partly confined conditions, i.e., lateral deformations can take place. Hence, the mechanisms involved in the oedometer test and in the *in situ* plate sinkage test are different (Figure 59.5).

Earl (1997) showed that for small deformations, the data from confined compression tests are similar to those obtained from plate sinkage tests (corresponding to the stress range 0 to ca. 300 kPa in Figure 59.5). It is believed that the precompression stress is identified within this range of deformation (Dawidowski et al. 2001). At greater deformations (corresponding to the stress range >ca. 300 kPa in Figure 59.5), however, the further downward movement of the plate is mainly caused by lateral deformation and not by compaction, whereas in a confined test, the deformation is caused by compaction (Earl 1997).

Therefore, all critical data from plate sinkage tests should be collected before the occurrence of any lateral soil deformation under the penetrometer plate (Dawidowski et al. 2001), i.e., only data associated with relatively small plate sinkage should be considered for determination of the precompression stress. Dawidowski et al. (2001) and Keller et al. (2004) measured precompression stress with a plate sinkage apparatus and an oedometer, respectively, on three different soils with textures ranging from loam to clay. They considered data

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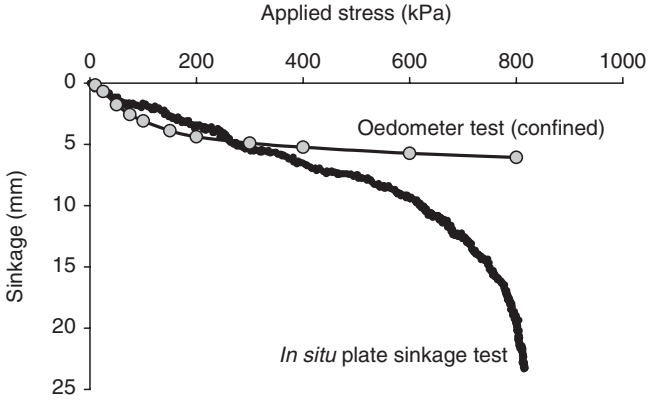

FIGURE 59.5. Experimental curves of axial (vertical) stress versus plate sinkage on a moist silty clay loam for confined oedometer test and *in situ* plate sinkage test; note that stress is plotted on linear scale in this figure in contrast to Figure 59.1 and Figure 59.4.

of plate sinkage <5 mm and found that the precompression stress values derived from the oedometer and the plate sinkage test did generally not differ significantly from one another.

59.5.2 Another Laboratory Method: Triaxial Cell Apparatus

The triaxial cell apparatus is used to measure soil mechanical properties that describe both the compressive and shear behaviors of soil. The former is described by C_s, C_c, and σ_p, while the latter is usually described by the Mohr–Coulomb parameters cohesion and angle of internal friction.

The triaxial cell apparatus allows for applying an axial vertical stress, σ_1 (by a piston) and a lateral confining stress, σ_3 (by hydrostatic pressure). Note that σ_3 is unknown in the oedometer test. Isotropic triaxial compression tests imply that $\sigma_1 = \sigma_3$. In a triaxial cell, the cylindrical soil specimen is enclosed by a rubber membrane and embedded between ceramic plates at the lower and upper faces to allow drainage.

Generally, oedometer tests are preferred to triaxial tests for engineering applications because they are less time-consuming and easier to use.

59.5.3 Range of Variation of Soil Compressibility Parameters

Typical values for precompression stress may vary from 30 to 150 kPa for moist soils, whereas compression indexes may vary from 0.05 to 0.35 (Lebert and Horn 1991).

Precompression stress and compression index have been measured on different soils and under various conditions. As mentioned elsewhere, the mechanical properties of soil are influenced by soil texture, organic matter content and composition, bulk density, soil water potential (water content), and soil structure. Consequently, several authors including Gupta and Larson (1982), McBride (1989), Angers (1990), Lebert and Horn (1991), McBride and Joosse (1996),Veenhof and McBride (1996), Défossez et al. (2003), and Imhoff et al. (2004) have proposed pedotransfer functions for the estimation of soil mechanical parameters from easily measurable soil variables such as soil texture, organic matter content, bulk density, and water content.

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Chapter 60
Field Soil Strength

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60.1 PRINCIPLES AND PARAMETER DEFINITIONS

Soil strength can occur in various forms or expressions, such as compressive strength, shear strength, and tensile strength, etc. There are many different soil strength measurement techniques, most of which originate from a specific applied soil test requirement. Soil tillage operation will often include a number of directional forces that the soil's strength resists until it breaks. The compressive strength resists tool insertion, the shear strength resists action of crushing soil by lateral forces, and the tensile strength resists the breakage of soil, which involves pulling it apart at the edge of tillage tines. While the ability of the soil to sustain traffic or support a building relates to compressibility and compressive strength, as discussed in Chapter 59, the soil strength associated with root penetration or tillage tool resistance can be conveniently ascertained by a penetrometer, which measures penetration resistance of a device inserted in the soil (most often a vertically oriented measure). The strength of soil to resist landslide and some forms of erosion, for example, is better expressed by techniques that explicitly measure the shear of the soil (a planar measure), such as standard sheargraph techniques.

The coverage in this chapter will be limited to two soil strength measurement approaches: cone penetration resistance and shear strength. Only specific cone resistance and shear strength methods that relate most strongly to manually operated *in situ* agricultural and environmental science evaluations will be discussed hereafter. For information on measuring tensile strength, we recommend the review by Dexter and Watts (2001).

60.2 PENETRATION RESISTANCE

60.2.1 Principles of Operation of Penetrometers

Penetrometers measure the force required to push or drive a device into a soil. The oldest penetrometers in agriculture could well have been animal hooves, where the depth of penetration advised the farmer whether a field was ''trafficable'' for grazing or cultivation. Essentially the principles of modern penetrometers follow the ''cruder'' soil penetration resistance indicators but with the added advantage of standardized measurements, which provide better repeatability and comparability.

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A penetrometer consists of a rod or shaft, which is pushed into the soil, usually vertically, using some device to measure the force required to insert the device to a desired depth. The end of the rod, or tip, engaging the soil is commonly conical or flat in shape. Similarly a number of force-sensing methods are used along with a variety of recording devices. Penetrometer data are typically reported as the resistance to soil penetration in terms of penetration force per unit area using units of pressure. For penetrometers using conical tips, the resistance pressure is often referred to as ''cone index'' (CI) and expressed in units of pascal (Pa), kilopascals (kPa), or megapascals (MPa), where $1\ \text{Pa} = 1\ \text{N m}^{-2}$. Most penetrometers use a standardized 30° circular cone with less than 700 mm^2 base area, which allows for manual as well as more mechanized probe insertion into most soil conditions.

Bengough et al. (2001) and Lowery and Morrison (2002) have given extensive and comprehensive reviews of soil penetrometers and penetrability, including background considerations that must be taken into account when undertaking soil penetration studies. Although numerous penetrometers are available, only general criteria for their selection have evolved. Increased data logging and smaller scale motorized drives have enhanced the opportunities for methodology studies comparing different penetrometers from which selection criteria can further evolve (Motavalli et al. 2003). The difficulty in defining objective criteria for penetrometer selection is complicated by (i) number of soil physical factors that influence penetration resistance in the soil and (ii) logistical and operational constraints to measurements (e.g., desired depth, crop damage, transport to in-field locations, measurement goals, cost, etc.).

Soil penetrometers can provide *in situ* data at depth in the soil profile without the need for soil disturbance. Obtaining equivalent soil mechanical measures in a laboratory setting would require major sampling, resulting in a destructive investigation of the soil body. Soil penetrometers fall into two basic categories, ''dynamic'' and ''static.'' Dynamic penetrometers are designed to be driven into the soil using the impact of a hammer or a falling weight. This type of penetrometer is used for highway pavement and roadbed evaluations, but few have automated data acquisition capabilities and are not widely used in soil science investigations. Static penetrometers are designed for insertion into the soil at a slow steady rate to avoid the need to include dynamic effects in the analysis. Only static penetrometers will be discussed in this chapter.

Soil Physical Factors Influencing Penetration Resistance

Soil penetrometers measure the force required to deform soil material, the nature of the deformation depending primarily on the shape of the penetrometer tip and on penetration depth. At the soil surface, the deformation is primarily shear, transforming gradually to a combination of shear and compression with increasing depth. This transition is exhibited as an increasing resistance with depth, where the transition depth is estimated to be between three and five diameters of the probe (Barley et al. 1965; Waldron and Constantin 1970). Beyond this depth, the deformation has been primarily explained using cavity expansion theory (Farrell and Graecen 1966; Vesic 1972). Farrell and Graecen (1966) have assumed compression to occur in two zones, an inner zone with plastic failure surrounded by elastic compression. In addition to shear and compression occurring in the soil there is also the force of friction on the cone/shaft. Thus a penetrometer records some combination of shear, compression deformation, and probe-to-soil friction. The combined effects expressed in

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the resistance readings, however, can be used as an indicator of soil strength. These factors, in turn, are influenced by the state of the soil physical conditions such as soil water potential, water content, soil texture, bulk density, structure, and clay mineralogy. The interaction and combination of these factors have a complex effect on subsequent penetration resistance readings. Hence penetration resistance measurements are seldom useful in isolation unless one is simply concerned with identifying the strength of a soil in relation to critical threshold values. However, in most cases, for effective use of penetrometer resistance to characterize soil behavior or soil treatment effects. concurrent independent measurement or control of these complicating factors will be necessary. This is the most important one for monitoring and comparing site conditions.

Tip Designs

The American Society of Agricultural Engineers has established an Engineering Standard, ASAE S313.3 Feb99, for the use of the soil cone penetrometer (American Society of Agricultural Engineers (ASAE) Standards 1999). This standard provides guidelines including purpose, scope, definition, test apparatus, procedure, and interpretation and reporting of data obtained by cone penetrometers. The standard adopts, as its model, a 30° circular stainless steel cone with a driving shaft of smaller diameter to minimize soil friction along the shaft. Penetrometer cones of this type are most common for soil-based activities.

As described by Lowery and Morrison (2002), the size of cone angle determines the relative portions of the cone resistance attributed to the two principal forces: (i) force to deform the soil by the wedge-action of the cone and (ii) soil-to-metal frictional force. For a given base diameter, a decrease in cone angle gives an increase in surface area, and thus an increase in the frictional force component. At an angle of about 30° there is a ''cross over,'' where for angles $<30°$, the cone resistance decreases as the angle increases, and for angles $>30°$ the cone resistance increases as the angle increases.

The cone diameter is an important consideration when measuring in well-aggregated or cracking soils. Cones with small base diameters may produce highly variable data when random measurements are made on soil aggregates, and in the interaggregate pore space or cracks that have lower resistance (Grant et al. 1985). Larger diameter cones may reduce these effects at the expense of greater deformation of aggregates (Jamieson et al. 1988), and potentially affecting measurements on large-scale structural units.

Penetration Rate

Penetration rate for penetrometers has not been standardized because of the potential interaction between rate and cone resistance, especially for finer soils (Freitag 1968). Moreover since soil water potential is a determining factor in soil strength (Busscher et al. 1997), any action to alter the soil water potential during penetrometer measurement potentially influences cone resistance. A cone advancing into soil causing local compression can change the soil water potential also. If the change in potential is not dissipated as quickly as the cone advances, this potential change can manifest as an error in cone resistance. It is important to use a slow, steady rate of penetration in order to compare soils and sites, and to document and record the rate used. The ASAE standard (American Society of Agricultural Engineers (ASAE) Standards 1999) recommends that the rate should not exceed 30 mm s^{-1}. Much slower rates of penetration have been used in other studies (Cockcroft et al. 1969;

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Waldron and Constantin 1970; Perfect et al. 1990) in an attempt to emulate plant root elongation rates, which are in the order of 2.78×10^{-4} mm s^{-1}. Such low rates are too slow for practical use in data collection except for specialized research.

Concurrent Measurement of Water Content and Penetration Resistance

Any soil will produce different cone penetration readings at different water contents (Busscher et al. 1997; Lapen et al. 2004). Therefore, water content is critical for interpreting penetration readings especially when comparative studies are made. In recent times, a large number of penetrometers have been developed to measure water content and cone resistance concurrently (e.g., Morrison et al. 2000; Young et al. 2000; Topp et al. 2003; Sun et al. 2004; Hummel et al. 2004). A variety of approaches to water content measurement have been utilized but no single approach has been shown to be superior. Hummel et al. (2004) used a standard cone modified to collect near-infrared reflectance from the soil passing the cone to estimate water content. Other approaches have used high-frequency electromagnetic wave propagation, including time-domain reflectometry (TDR) as a basis for water content measurement. Young et al. (2000), and Topp et al. (2003), used time-domain transmission (TDT) by inserting a transmission line on the periphery of the shaft behind the penetrometer cone (Figure 60.1). The helical wrapped transmission line used by Topp et al. (2003) had an

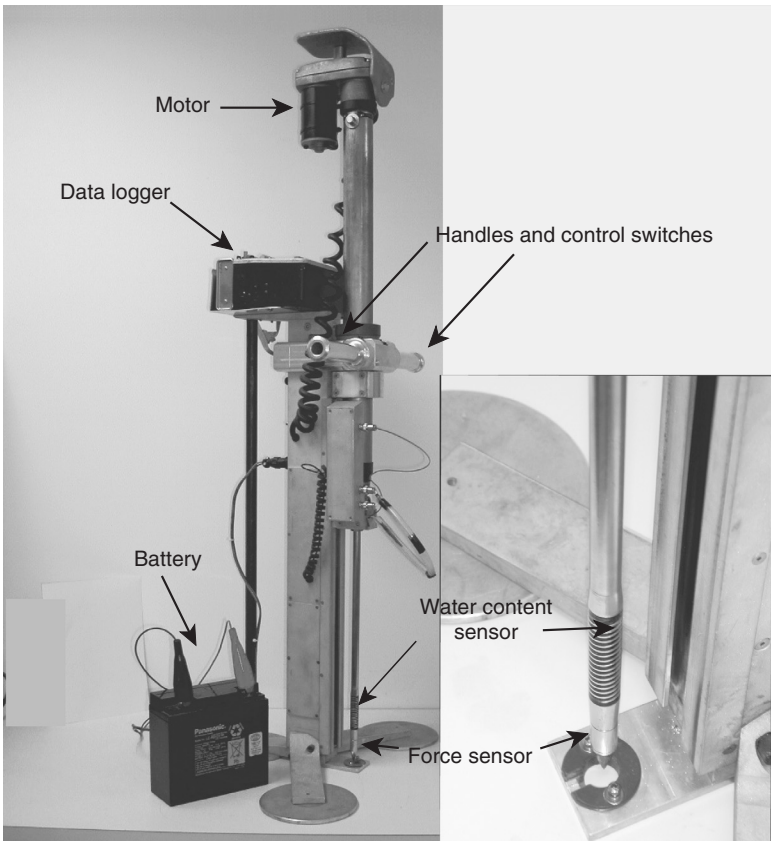

FIGURE 60.1. A motorized portable penetrometer for measuring both cone resistance and water content. (From Topp, G.C., Lapen, D.R., Edwards, M.J., and Young, G.D., *Vadose Zone J.*, 2, 633, 2003. With permission.)

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advantage over Young et al. (2000), in that the water measurement zone was reduced to 6 cm along the shaft while maintaining a transmission line length of 30 cm. The reported water content resolution of this shaft-based helical TDT system was ± 0.02 m^3 m^{-3}, which is equivalent to the resolution of a TDR. Morrison et al. (2000) equipped a cone penetrometer with a small TDR transmission line for measuring water content at the cone surface, in which the water content sensor was that described by Starr et al. (2000). Sun et al. (2004) have used a capacitance- or impedance-type sensor at the base of the cone to estimate water content. As these combination penetrometers are relatively new, they have not yet been used extensively to allow ranking or comparative assessments. The principle of operation of some of the above-mentioned electromagnetic sensors is covered in detail in Chapter 70.

60.2.2 Instrumentation

Hand-Push Portable Cone Penetrometers

Portable cone penetrometers are hand-push devices that are about 1.2 m in height allowing for penetration depths to about 0.8 m (some models with rod extensions can provide measurements deeper in the soil). The cones are smoothly machined stainless steel and most commonly have the ASAE recommended 30° cone angle. The cone base and corresponding rods will be usually available in two sizes: (i) 9.5 mm diameter rod with a 12.8 mm diameter cone and (ii) 15.9 mm diameter rod with a 20.0 mm diameter cone for use in ''soft'' soils. The force-sensing devices are located at the upper end of the shaft and use a variety of sensing techniques such as proving ring and dial gauge and strain gauge-based load cells. Depth sensing is achieved by methods such as graded markings on the rod, laser reflectance from the soil surface, a potentiometer, and more. If data logging and data manipulation equipment are employed, they are typically mounted above the load sensors. In most units, two hand-grip handles are conveniently attached to opposite sides of the enclosure for the electronic components. Between the handles on the upper side of the electronics is usually the instrument panel and data display. The insertion rate is operator controlled. Portable cone penetrometers are commercially available from ELE/Soiltest International, Inc., Loveland, Co, USA; Eijkelkamp Agrisearch Equipment BV, Giesbeek, The Netherlands; Spectrum Technologies, Inc., Plainfield, IL, USA; Agridry Rimik Pty Ltd., Toowoomba, QLD, Australia.

Motorized Portable Cone Penetrometers Measuring Water Content

Soil water content is one of the most important factors influencing cone penetration measurements, and for most soil-based applications, it is a mandatory measurement. Penetrometers with simultaneous water content measurement features have developed from portable cone penetrometers and have two added features. The first is to measure water content and the second is to control the rate of penetration. To achieve these improvements, it requires a stand and a frame to support the motor and guide the penetrometer, resulting in a large unit, which approaches the limit of human portability. Nevertheless, the operational advantages of such combination penetrometers warrant their inclusion here.

The general operation of a combination penetrometer with both water content and cone resistance measurements is described by Young et al. (2000) and Topp et al. (2003). The penetrometer system (Figure 60.1) uses a lightweight, 12 V electric motor-driven screw jack assembly to provide a constant velocity for probe insertion of approximately 28 mm s^{-1}. The length of travel is 400 mm. The drive mechanism is mounted on a vertical supporting frame made of lightweight material, which also houses the sensor electronics, data logger, and

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motor control switches. The vertical frame is supported by three horizontal legs on which the operators stand to provide a load to hold the equipment in place during operation. The power for the whole system, including the drive motor, is supplied by a 12 V gel-cell battery, which is carried in a backpack. In Topp et al. (2003), the water content sensor on the shaft, a helical transmission line, is connected between 50 Ω coaxial cables to transmit and receive a high-frequency signal along the helix from which the travel time of the signal is used as a measure of water content of the surrounding soil (Figure 60.1). The force sensor at the cone was described previously (Adams et al. 2000).

Sun et al. (2004) described a similar combination penetrometer, but with a shorter capacitance-type sensor for water content. Apart from being at an early stage of development, and less readily portable, the Sun et al. (2004) penetrometer has similar operational properties to that of Topp et al. (2003).

60.2.3 Procedures

The procedural details for the use of penetrometers depend on a number of factors: (i) intended use for the data, (ii) type and capability of the penetrometer, and (iii) soil or field conditions at the time of measurement.

Cone Penetrometers

Cone resistance data are highly variable, necessitating carefully chosen levels of replication to assure high-quality data. Ideally, the degree of replication should be made on the basis of a presampling survey from which one obtains sample variance and mean. From these, the number of replicates required to attain the desired confidence level can be calculated. Cone penetrometers are usually used to indicate conditions within soil profiles. As surface operations on the soil such as tillage and traffic affect the strength distribution in the soil profile, the location of measurement and degree of replication at a field site are the first considerations.

1. Adopt criteria for location of measurement, so that replicated locations can be chosen consistently and in relation to the treatment conditions and objectives of the study. For example, if a comparison of plant row position and wheel traffic are part of the study treatment, locations should be chosen as mid-way between rows in wheel-tracked and/or nontracked rows. In another case, the choice may be only ''in plant rows.''

2. Layout the field sampling program so that replication, data labeling, and site location criteria are specified prior to the initiation of the measurements.

3. Schedule the field measurements to optimize the quality of the data obtained. As cone resistance is highly dependent on water content, it is advisable to aim for simultaneous measurements of water content. In cases where water content cannot be measured as frequently as cone resistance, it is recommended that resistance testing be done at specified water content, such as at ''field-water capacity'' or some equivalent condition that occurs repeatedly either seasonally or within a season.

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4 Set up penetrometer to begin data collection. The details of this step depend on the type of penetrometer in use. For gauge type and similar manual penetrometers, this amounts to setting the force indicator to zero. For data logging penetrometers, this step involves initiating the data logger and resetting the data labels.

5 Push the penetrometer into the soil at a uniform rate not to exceed 30 mm s^{-1}. For hand-push penetrometers, the rate control is the responsibility of the operator and some commercial devices have aids to indicate when the desired rate is exceeded. Unsteady rates of insertion often occur as an operator forces the penetrometer tip past occasional harder layers or large pores. This step is more readily achieved satisfactorily with motorized devices.

6 Withdraw the cone from the soil, and wipe it clean, if necessary.

7 Take any necessary steps to ensure that the data have been recorded or retained on the data logger. For fully manual penetrometers, only extreme or limited values of resistance can be recorded, but most data logging penetrometers will automatically carry out this step.

8 Reset the penetrometer and return to step 4 to continue with the predetermined replication schedule for the selected location.

9 Retrieve and download the data as required to meet the objectives of the study.

60.2.4 Calculations and Data Handling

As there is no consistent or standardized protocol for the acquisition or analyses of penetration resistance data, only general considerations will be dealt with here, leaving it to the operator to ensure that reported data have been retrieved and processed properly. Proper calibration of the instrument is required and periodic testing of the stability of the calibration before, during, and immediately following field use is recommended.

Typically calibration applies to three aspects of penetrometry: (i) depth recording and resolution, (ii) force or resistance, and (iii) water content for instruments that include this measurement. In general, the depth indicators and sensing devices are often precise and stable enough to allow infrequent calibration. However, periodic examination of their accuracy is certainly recommended.

Adams et al. (2000) described a method for calibrating the force sensors in the laboratory. Such calibration is critical and should be conducted routinely under controlled laboratory conditions. Methods vary according to design of the device.

Topp et al. (2003) developed in-field procedures for checking the calibration of the force and water content sensors. The general approaches used are applicable to any sensor, but the details will need to be altered depending on specific sensor requirements. Further details on calibration may be obtained from Topp et al. (2003).

Penetrometer calibration is usually achieved using a series of weights acting as loads exerting different forces. The exterted force (N) $= m$ (kg) $\times$ g (9.81 m s^{-2}), where g is the acceleration due to gravity and has a constant value. Penetrometers may record and store data

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in different forms, depending on the data-handling capability of the devices used. In cases where resistance is not measured in the units of pressure, it is important that the data are converted to pascals (Pa). Fortunately, most commercially available automated units have internal conversion that present the final values in Pa. The area used in the conversion of force to pressure is the cone base area or the cross-sectional area of the flat-tipped probe of the penetrometers, hence it is important that the tip used is correctly matched for the conversion calculation.

No specific guidelines exist for penetrometer data analysis; therefore, the choice of analyses will depend upon the requirements of the overall study for which the penetrometer data form a component.

60.2.5 Comments

As mentioned in the previous sections, field-based penetration resistance measurements can be highly variable even within similar soil types. Stones, vegetation, soil structure, texture, and trafficking, for instance, contribute to the variability, notwithstanding operator variance. Penetration resistance data have been found to exhibit spatial independence at less than 1 m (O'Sullivan et al. 1987). Lapen et al. (2001) showed that for a clay loam soil under grass, the values had a variable range of spatial dependency based on the direction of field traffic, where shorter distances in spatial correlation were found perpendicular to dominant trafficking patterns. Recommendations on the minimum number of required measurements to achieve desired confidence intervals of averaged values have been put forward (e.g., Bengough et al. 2001). However, such recommendations should not be taken as a strict rule, but rather as a guide, particularly when the required minimum number of measurements cannot be achieved due to logistical or cost constraints. In all situations, a key rule of thumb for penetrometer operators is to acquire as much information about site characteristics as possible to help inform spatial sampling strategies, bearing in mind that there is no single strategy that will meet all requirements for all situations.

Any study attempting to make relative comparisons between soil treatments, for instance, must take into consideration soil water content on the measured cone resistance values. Ideally, the most preferable option is the use of a combination water content/penetrometer. However, if such an instrument cannot be used, an independent measure of soil water content should be made close to the measurement site. In most situations, the relationship between soil water content and cone resistance is negatively correlated. Taking account of this relationship can minimize errors associated with strength–water content interactions, which might otherwise ''mask'' soil management effects on strength properties. Busscher et al. (1997) proposed the general equation (Equation 60.1) to standardize penetration data to a common water content to minimize confounding effects:

$$C_c = C_o + \frac{dC}{dW}(W_c - W_o) \tag{60.1}$$

where C_c is the corrected cone index, C_o the original cone index, W_c the common water content to which the cone indices are being corrected, W_o the original water content of C_o, and dC/dW is the first derivative of any one of the following equations (Equation 60.2 through Equation 60.4) that best fitted a set of cone index values to water content:

$$C = aW^b \tag{60.2}$$

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$$C = a(1 - W)^b \tag{60.3}$$

$$C = ae^{bW} \tag{60.4}$$

where C is the cone index (MPa), W the soil water content on a dry weight basis (g g^{-1}), e the base of the natural logarithm, and a and b are empirical parameters.

In some cases, depending on soil treatments/conditions, the water content vs. cone resistance relationship may vary temporally, for example, soil consolidation over time may change the water content relationship in addition to the strength differences related to different soil treatments or soil condition. For example, Lapen et al. (2004) found temporally consistent water content vs. penetration relationships for nontilled, clay loam soils, but for conventionally tilled soils, the relationships were temporally variable for nearly 3 months after spring planting. Such findings underscore the importance of acquiring as much site information as possible prior to engaging in field monitoring programs.

60.3 SOIL SHEAR STRENGTH

The shear strength of soil is an important consideration for the design of foundations, retaining walls and roadways in civil engineering applications. In agricultural applications, shear strength is expressed during wheel traction and when tillage tools act to fracture the soil. Shear strength is defined as the strength of the soil, just as it ''fails'' or rather when the soil is unable to resist deforming forces applied to it. In the field, most of the forces applied to soil are multidirectional, making it difficult to define the forces that cause the soil to only shear. As a result, there are only a few soil strength methods that are directly applicable to measure soil shear strength in the field. Most shear strength measurements are described for laboratory samples, performed either on remolded soil, or structurally intact soil blocks, or cores. Being a measure of soil strength, shear strength varies with soil water content or more specifically the soil water potential. In general, soil strength increases as the soil dries or the water potential decreases. In a detailed study of laboratory measurement of shear strength of unsaturated soils, Fredlund and Vanapalli (2002) summarized the current state of the research on the importance of soil water potential on soil shear strength. They have also provided many references to a variety of texts and standard guidelines where methods for measuring shear strength are described for engineering specifications.

Field measurement of shear strength has progressed more slowly and few techniques are available. Lloyd and Collis-George (1982) described four types of shear that can occur in structured field soil depending on the relative strength of aggregates and other structural features. Such fracturing detail is difficult to capture and measure after sampling and transport to the laboratory. Lloyd and Collis-George (1982) described a torsional shear box for field measurement of shear strength under near-zero load, as a best estimate of the contribution of cohesion to soil shear strength. A hand-held shear vane, sometimes called a torvane, also measures zero-load shear strength or cohesion at the cylindrical surface enclosing the outer limit of the vane blades (New Zealand Geotechnical Society, Inc., 2001). A similar measurement can be made at the soil surface with a similar torsion head attached to a grouser where vanes are perpendicular to a circular disk and aligned along radii. Some torvane kits include both surface vanes of different diameters for differing strength soils and axial vanes of differing dimensions for soils having different strengths. Surface vane measurements are somewhat difficult as measurement is made at zero-load, which is hard to maintain consistently while applying the shearing torque. In this section, the method

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of using the Cohron portable sheargraph, a torsional shear device, for *in situ* measurement of field shear strength (Cohron 1963) is described. Although similar in principle of operation to the torsional shear box and the surface shear vane, the Cohron sheargraph adds the advantage of measuring the normal stress or load applied during shear.

60.3.1 Principles of Measurement

Soil shear strength arises from two characteristics of the soil body: soil cohesion and internal friction. Cohesion in soil arises from the bonding and attraction between particles as well as bonding caused by water films. The resistance that occurs when soil particles interlock and rub against each other during shearing is the frictional component. The shear strength is usually represented as

$$\tau = c + \sigma_n \tan \varphi \tag{60.5}$$

where τ (kPa) is the shear strength, c (kPa) cohesion, σ_n (kPa) the normal stress, and tan φ the friction coefficient, in which φ is the angle of friction. Equation (60.5) is based on an assumed failure mode defined by the Mohr failure envelope and a linear relationship between shear and normal stresses for the failure surface (Ayers 1987). In practice c and tan φ are empirically obtained by measuring the force required to cause shearing of the soil (τ) when subjected to a sequence of varying normal stress (σ_n). From a linear regression of τ against σ_n, both cohesion (c) and the angle of friction, as tan φ, can be determined from the τ-intercept and the slope of the regression, respectively.

The Cohron sheargraph is a torsional shear device used to measure soil strength *in situ*. As with all field torsional shear devices, this device also suffers from the disadvantage that the stress distribution on the shear surface and the exact geometry of the shear plane cannot be determined as well as in laboratory methods. However, the usefulness of this device outweighs any disadvantages (Kirby and Ayers 1993). One of the less attractive operational features of the Cohron sheargraph is the mechanical recording of a graph and subsequent graph digitization (see Section 60.3.3). In addition, there is need to determine water content by the gravimetric method. Recently, we have been modifying the sheargraph to overcome these two difficulties by incorporating TDR measurement of water content and data logging of the stress data directly. These modifications are presented below as an alternative approach under the name of shearlogger.

Ayers (1987) has reported a large number of sheargraph measurements to examine the relationships among cohesion, friction, water content, and density. Further, Kirby and Ayers (1993) have interpreted such measurements in terms of critical state soil mechanics concepts.

60.3.2 Equipment and Supplies

The Cohron sheargraph is available only from custom-designs following the description given by Cohron (1963). Kirby and Ayers (1993) provide a schematic diagram, reproduced here in Figure 60.2, to illustrate the essential components.

The shear head is a 40 mm diameter circular disk with six grouser blades (10 mm depth by 1 mm thick), extending 10 mm perpendicular to the disk, along six equally spaced radial axes

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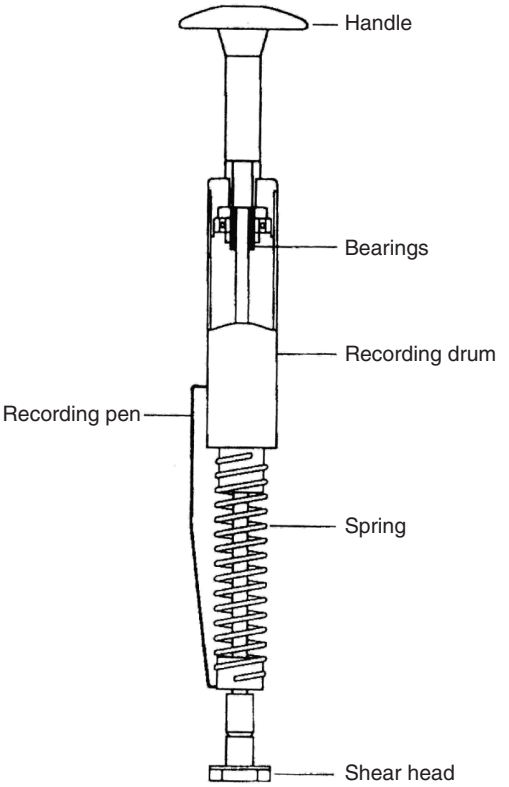

FIGURE 60.2. Schematic drawing of Cohron sheargraph. (Kirby, J.M. and Ayers, P.D., *Soil Till. Res.*, 26, 211, 1993. With permission.)

and into soil. For softer soil, a larger shear head (80 mm diameter) is used. Thus the shearing surfaces (the areas of soil embedded in the shear head) for the 40 and 80 mm diameter heads are 1257 and 5027 mm^2, respectively.

The coil spring allows the recording of both the applied normal stress (the downward force) and the shearing resistance (the torque). A coil spring of 35 mm inside diameter having approximately 10 coils of 3 mm (8 gauge), wire in a 150 mm length provided a maximum normal stress of 300 kPa on the smaller (40 mm diameter), shear head (equivalent to a force of approximately 375 N).

The recording pen consists of a spring-loaded pencil holder such that an inserted pencil records the movement of the spring on user-provided graph paper wrapped around the recording drum. An alternative is to use a combination of a pressure-sensitive graph paper and a spring-loaded scribing point. The handle is used to apply downward forces manually during measurement.

Other required equipment, such as an Instron loading machine (Ayers 1987), is for the calibration of the coil spring both in the axial and torsional or rotational directions, so that axial compression and angular rotation can be converted to units of force.

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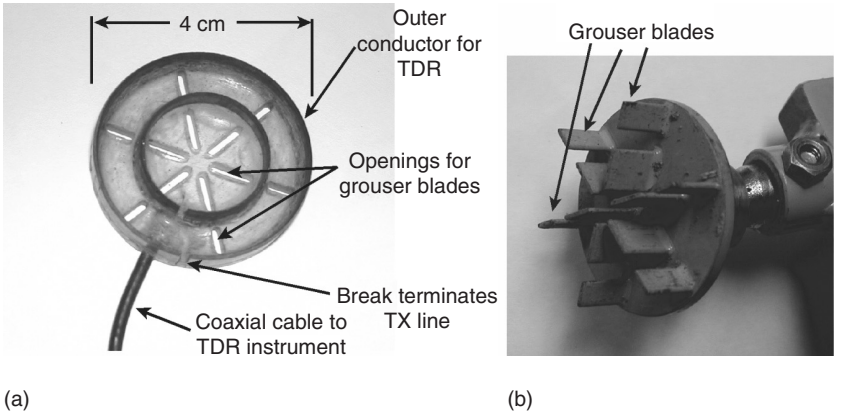

FIGURE 60.3. (a) Water content sensor to be used in conjunction with the Cohron sheargraph or shearlogger, (b) shear head showing grouser blades which insert into and through openings in (a) after the measurement of water content has been completed.

For greater automation during strength measurements, the Cohron sheargraph has been modified in two important ways. One was to provide for the simultaneous measurement of soil water content using TDR and the other was to automate the recording of normal and shear stresses. For use with the TDR, the shear head was modified to include a pair of circular concentric blades of similar depth to the grouser blades and act as TDR transmission (TX) lines (Figure 60.3a). The blades of radii 10 and 20 mm are set in place by a 3 mm thick layer of epoxy resin. The termination of the TX line is achieved by a 2 mm vertical gap in each concentric blade. On each side of the gap, a 5 pf capacitor connected between the two blades provided clear zones in the TDR signal that indicated the ends of the TX line. Figure 60.3a shows slots in the plastic through which the grouser blades of the shear head (Figure 60.3b) are inserted for shear measurements. TDR measurements have been made using the TRASE model instruments (Soilmoisture Equipment Corp., USA) by the authors but other TDR instruments can also be used.

The second modification for automatic recording of stresses was the addition of two strain gauge load cells one to record normal stress and the other to record shear stress, both placed between the shear head and the spring assembly (Figure 60.4). Although the load cell modification minimized the need for the coil spring, the spring was retained to facilitate an estimation of applied stresses by the operator. The voltage signals from the load cells were recorded by a CR510 datalogger (Campbell Scientific, Inc., Canada). Load cells were an S-shaped sensor (0–100 lb, i.e., 45.4 kg load range (Intertechnology Ltd., Canada)) for normal stress and a miniature round beam (50 lb, i.e., 22.7 kg load capacity (Artech Industries, Inc., USA)) for shear stress. As the graphing function was no longer needed, the pencil holder assembly was removed and replaced with two parallel plastic bars along the sides of the arm to which the pencil holder was mounted to transmit the applied torque from the handle to the load cell assembly without using the spring to provide the torque. When the spring was used without the plastic bar restraints, there was a tendency at peak shear stress for the spring to go rapidly and precipitously to the lower stress level. During this rotational ''snap'' of the spring, it was difficult for the operator to maintain consistent normal stress and for the recording of the rapid transition to a lower shear stress value without the restraining bars.

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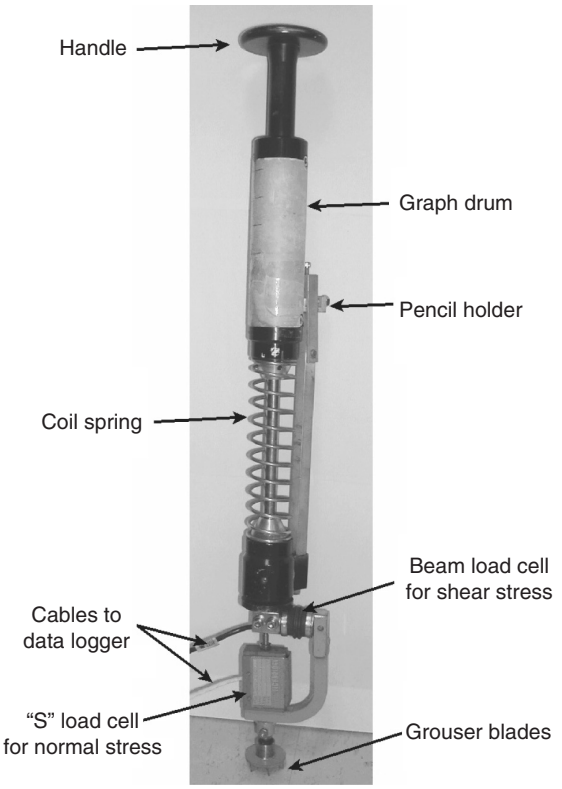

FIGURE 60.4. Shearlogger or Sheargraph as modified to use load cells for recording normal and shear stresses.

60.3.3 Procedures

Both the sheargraph and the shearlogger are custom-designed following Cohron's (1963) description and as a result the procedures are similar. As the shearlogger is more efficient and easier to use, it will be described as the primary method with indicative statements given for use of the sheargraph.

Cohron sheargraph or shearlogger measurements are made on a 50 × 50 mm flat surface for the small shear head; a larger area is used for the large head. The soil surface for measurement is prepared by gently clearing vegetation and producing a level soil surface. Deeper levels in the soil profile are accessed by excavating stepped levels down the side of the soil pit.

The following procedure is carried out for measurement at each location:

1. Water content is measured first. Insert the water content sensor blades into a flat soil surface until the resin (plastic) top is just in contact with the soil surface.

2. Record the water content using the TDR instrument (see Chapter 70 for details).

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3 Insert shear head grousers through the openings in the plastic top of the water content sensor, and into the soil below, until the top of the shear head touches the top of the water content sensor (Figure 60.3). Gently clear the soil around the outside of the water sensor using a penknife or spatula.

4 Initiate data logging from the load cells on the shearlogger.

(a) For the sheargraph, install and set graphing chart and pencil for correct alignment and positioning for ''zero''-load condition.

5 Choose three to five levels of normal stress to be applied to the soil, achieved by applying a vertical downward load (pushing) on the handle of the shear-graph/logger. Ayers (1987) used four levels of normal stress: 0, 20, 35, and 70 kPa on repacked soils in the laboratory. The authors used five levels of normal stress on field soils: 60, 100, 150, 200, and 250 kPa (exemplary data for the lower four levels are shown in Figure 60.5).

6 The procedure for applying normal stress and shearing force follows the sequence indicated by numbered arrows in Figure 60.5. The normal stress is increased from zero to one of the preselected values in step 5 above, while the shear stress is zero (following path I in Figure 60.5). This amounts to pressing the handle downward without rotation.

7 The normal stress is maintained constant, while the shear stress is slowly increased by rotating the handle as indicated by the shear head tending to rotate (going along path II in Figure 60.5) until the soil fails. At failure, the point of

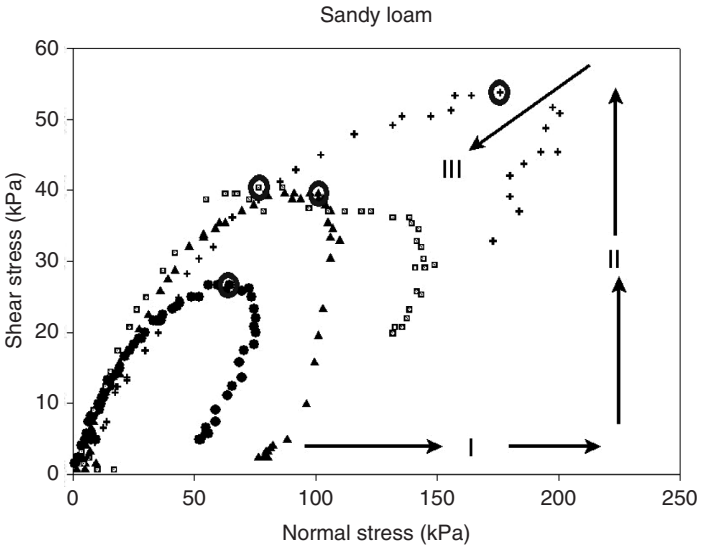

FIGURE 60.5. Normal–shear stress paths recorded during four measurements in a sandy loam soil. The numbered arrows show the sequence along which the normal and shear stresses are applied during each measurement.

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maximum shear stress for the soil is reached beyond which shear stress reduces. This step will generally require considerable practice as most operators cannot maintain constant normal stress during rotation of the handle, and the data will show a ''wobbly'' path to maximum shear stress.

8 After failure, normal and shear stresses are decreased together until the normal stress is returned to zero following path III in Figure 60.5. The handle is raised decreasing the normal stress, and the shear head will tend to rotate to decrease the shear stress, which should be allowed by slight rotation of the handle in the direction indicated by rotational tendency of the spring. This completes the shear and normal stress data collection for this step, indicating data logging should stop. Generally three or more replicate measurements should be made at each selected stress level.

(a) During decreasing normal stress level on the sheargraph, the shear head will generally rotate back decreasing the shear stress simultaneously. The graph traced out on the recording drum will consist of the data set for one shear measurement. The data set will depict a closed loop of increasing and decreasing stresses indicating three stages of stress application as shown in Figure 60.5.

9 When using the sheargraph, the water content is determined after shear measurement by collecting soil samples from the shear head and acquired by gravimetric determination.

10 For both shearlogger and sheargraph, it is advisable to collect soil cores adjacent to the measurement site to determine *in situ* bulk density.

11 Download data from the datalogger and apply force sensor calibrations if this was not incorporated in the data logging procedure.

(a) Graph from sheargraph in steps 6, 7, 8(a) is digitized using a method of choice. Generally, this would involve optical scanning to digitize the image and the values converted to stress values based on the calibration information of the coil spring.

12 Plot normal and shear stress patterns as shown in Figure 60.5 and proceed to Section 60.3.4 for calculations.

60.3.4 Calculations and Data Handling

The calculation and data handling are the same for both the Cohron sheargraph and shearlogger as both produce similar data. With data from both devices, it is possible to derive more information than only the maximum shear stress at soil failure. The intent of the procedure in this section is to acquire two further parameters, cohesion and angle of friction, from the series of normal and shear stress data for a soil. As water content affects shear strength, it is important that similar soils at similar water contents are considered together. Thus water content measurement is one basis for grouping data for initial analysis.

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1. Plot the normal stress vs. shear stress data to produce curves similar to those in Figure 60.5.

2. From each curve (similar to Figure 60.5), obtain the point of maximum shear stress and the normal stress at which the maximum shear occurred. The circled data points in Figure 60.5 indicate the authors' choice for the data plotted in the figure.

3. Data set of maximum shear stress (τ) vs. normal stress (σ_n), obtained from Figure 60.5, are fitted with a linear regression that satisfies Equation (60.5) from which cohesion (c) and coefficient of friction (tan φ) or friction angle (φ) are obtained from the intercept and slope, respectively.

4. Table 60.1 presents regression results for soils with two different textures. The clay loam data were taken before and after a small rain shower and hence are shown at two water contents.

5. Clay loam soil showed brittle failure in some tests, for example, the solid line data in Figure 60.6. Data such as the solid line in Figure 60.6 may be interpreted for two types of shear strength, referred to as peak, and ultimate strengths (Kirby and Ayers 1993). The peak shear strength is given from the maximum shear stress before the large decrease to a region of lower slope shear stress decline, from which ultimate shear strength is obtained. Although regression parameters are presented in Table 60.1 for peak and ultimate shear strengths (Kirby and Ayers 1993), the detailed procedures are not included here.

6. Water content as measured by the shearlogger is useful for estimating the dependence of shear strength on water content, much as shown by Ayers (1987).

60.3.5 Comments

The data presented above have not been compared or validated against other methods of measurements. The normal and shear stress curves follow similar paths to those shown by Kirby and Ayers (1993) in their laboratory studies. In Figure 60.5, for the sandy loam, maximum shear stress occurs over a range of normal stress (i.e., the curves flatten at the maximum shear stress). Although a range of nearly equal normal stresses were recorded at the maximum shear stress, the authors have used the maximum normal stress for the regression results in Table 60.1. For the clay loam (Figure 60.6), there is a better definition of maximum shear stress, thus the choice for peak stress and corresponding normal stress is more straightforward. The clay loam graphs represent three replicates at the same water content (0.14 $m^3\ m^{-3}$), and some

TABLE 60.1 The Regression Parameters: Cohesion, Angle of Friction, and Water Content (WC) with Standard Deviation (St. Dev.) of Soils from Field Shearlogger Tests

	Cohesion (kPa)		Friction angle (°)		R^2		WC	Standard
Soil	Peak	Ultimate	Peak	Ultimate	Peak	Ultimate	($m^3\ m^{-3}$)	deviation
Sandy loam	9.7	—	12.8	—	0.612	—	0.216	0.027
Clay loam	24.5	7.3	6.7	10.4	0.496	0.824	0.140	0.017
Clay loam	16.3	5.9	18.4	14.4	0.846	0.679	0.207	0.018

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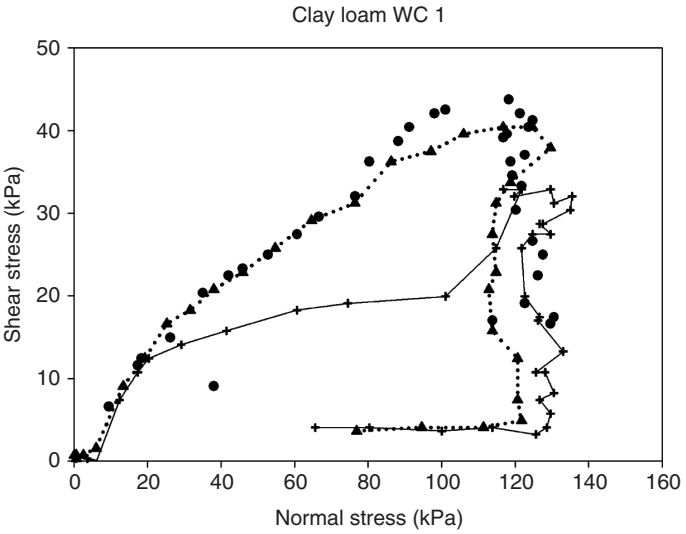

FIGURE 60.6. Normal–shear stress paths recorded during shear measurement in a clay loam soil at low water content. The three measurements are replicates with a targeted maximum normal stress of 120 kPa. The solid line data set indicates both peak and ultimate shear processes have occurred. Note the ''wobbly'' path rising from 120 kPa indicating the difficulty of holding a constant normal stress while applying increasing shear stress.

brittle failure can be detected in the replicate indicated by the solid line data in Figure 60.6. This is not seen in the other two replicates where more plastic failure has occurred.

The difficulty of maintaining constant normal stress as shear stress is increased is evident from Figure 60.6 where, although all measurements were intended to be made at a set normal stress of approximately 120 kPa, each data set showed considerable wobble ($\pm$7 kPa), which tended to increase as the shear stress increased and approached the maximum shear stress at which shearing occurred.

Although the Cohron sheargraph provides useful information in measuring soil shear strength (Ayers 1987; Kirby and Ayers 1993), it has had little use in the last 40 years. The technique involved a high level of operator tedium where acquired data had to be manually digitized to obtain graphical outputs, and the need to work with small number of soil samples, often restricted by gravimetric soil water measurement facilities and labor. The modifications to the sheargraph (shearlogger) presented in this chapter have overcome some of the tedious elements by using TDR to measure water content, and by adding load cells to allow logging of data digitally. This represents considerable time saving, in that, with the present shearlogger, and water content sensor, each stress path determination, and corresponding water content measurement took an average of 6 min in the field for a single operator, and the data collection was automatic and in digital format. Without the modification, using the graphical data from the recording drum, and manual gravimetric sampling for water content required almost twice the time in the field, and further laboratory procedures to get data into numeric format. The precision of the data obtained from the sheargraph, and the shearlogger is comparable and a more definitive evaluation of data quality and operational details of the shearlogger is currently underway.

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